Priorities for tuberculosis research

A report of the Disease reference group on TB, leprosy and Buruli ulcer

World Health Organization

TDR For research on diseases of poverty

UNICEF • UNDP • World Bank • WHO

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Cover picture: WHO/Stop TB

Design and layout: Lisa Schwarb, Lausanne

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Abbreviations and acronyms

ACF	Active case-finding
ADME	Absorption distribution metabolism excretion
ARI	Annual risk of infection
ART	Antiretroviral therapy
BCG	Bacille Calmette–Guérin
BSL	Biosafety level
CFSE	Carboxy-fluorescein succinimidyl ester
CYP	Cytochrome P
CPT	Co-trimoxazole preventive therapy
DOTS	Recommended international tuberculosis control policy
DRG	Disease reference group
DR-TB	Drug-resistant tuberculosis
DST	Drug susceptibility testing
DS-TB	Drug-sensitive tuberculosis
EBA	Early bactericidal activity
ECF	Enhanced case-finding
EMEA	European Medicines Agency
EPI	Expanded Programme on Immunization
FDA	Food and Drug Administration (US)
FDC	Fixed-dose combination
FM	Fluorescence microscopy
GCP	Good clinical practice
GLP	Good laboratory practice
HAART	Highly active antiretroviral therapy
HBCs	High-burden countries
HBHA	Heparin-binding haemagglutinin
HIV	Human immunodeficiency virus
HCW	Health-care worker
IC	Infection control
ICS	Intracellular cytokine staining
ICF	Intensive case-finding
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
IFNγ	Interferon-gamma
IGRA	Interferon-gamma release assay
IL	Interleukin
INH	Isoniazid
IND	Investigational new drug
IPT	Isoniazid preventive therapy
IRIS	Immune reconstitution inflammatory syndrome
LAM	Lipoarabinomannan
LAMP	Loop-mediated isothermal amplification
LED	Light-emitting diode
LPA	Line-probe assay

LTBI	Latent tuberculosis infection
MDG	Millennium Development Goal
MDR-TB	Multidrug-resistant tuberculosis
MODS	Microscopic observation drug susceptibility
MTB	Mycobacterium tuberculosis
NAAT	Nucleic acid amplification test
NCE	New chemical entity
NGO	Nongovernmental organization
NNRTI	Non-nucleoside reverse transcriptase inhibitor
NRA	Nitrate reductase assay
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase chain reaction
PI	Protease inhibitors
PITC	Provider-initiated testing and counselling for HIV
PLHIV	People living with HIV
PMTCT	Prevention of mother-to-child transmission of HIV
POC	Point of care
PPD	Purified protein derivative
RCT	Randomized controlled trial
R&D	Research and development
SOP	Standard operating procedure
TAG	Treatment action group
TB	Tuberculosis
TDR	Special Programme for Research and Training in Tropical Diseases
TLA	Thin-layer agar
TNF	Tumour necrosis factor
TST	Tuberculin skin test
UV	Ultraviolet
VCT	Voluntary counselling and testing
WHO	World Health Organization
XDR-TB	Extensively drug-resistant tuberculosis
ZAMSTAR	Zambia–South Africa TB and AIDS Reduction
ZN	Ziehl–Neelsen

Executive summary

Background: Tuberculosis remains an important global health problem. Although a number of important advances in its diagnosis and treatment have been made, further research is required to accelerate progress towards meeting the Millennium Development Goals and the goal of the Stop TB Partnership to eliminate tuberculosis as a global health threat by 2050. Tuberculosis is a priority tropical disease for the Special Programme for Research and Training in Tropical Diseases (TDR). As outlined in TDR's 10-year vision and strategy, Disease reference groups are crucial to the programme's stewardship mandate for acquisition and analysis of information on infectious diseases of poverty. TDR therefore commissioned the Disease reference group (DRG) for tuberculosis, leprosy and Buruli ulcer to provide an expert perspective on the current status of research on tuberculosis, to identify critical research gaps and to prioritize areas of research. The report identifies research priorities for tuberculosis in the following thematic areas: epidemiology and control; health systems and operational research; case-finding and infection control; HIV-associated tuberculosis, vaccines, new drugs and diagnostics; and social science and gender.

Methodology: Technical experts provided an overview of the current status of tuberculosis research and identified gaps and research priorities in each of their respective thematic areas. A Delphi technique was then used to select the top 10 areas of research. In the first stage, at least 10 research priorities were identified for each thematic area. In the second stage, the research priorities were compiled into a single list and distributed by e-mail to the DRG. Each member of the DRG then selected 20 research priorities from the combined list, without providing justification for their selection. The 20 priorities from each member were then again compiled into a single list, reviewed and edited so that duplicate priorities were removed and similar priorities merged. In the third phase, a face-to-face meeting was held to identify the final 10 high-level areas for tuberculosis research.

Results: The following high-level research areas were identified:

1. Increase understanding of the pathogenesis of tuberculosis to fuel discovery of drugs, vaccines and diagnostics.
2. Improve diagnostics for infection and disease, especially point-of-care tests.
3. Develop improved treatment and prevention regimens (based on current and new drugs).
4. Develop novel vaccines and optimize current vaccines.
5. Identify and validate biomarkers that facilitate development of vaccines, diagnostics and drugs.
6. Evaluate and optimize strategies to improve case-finding and reduce barriers to treatment.
7. Optimize implementation of preventive therapy for TB, including drug-resistant tuberculosis.
8. Evaluate and optimize new and current strategies to quantify, prevent and minimize disability and stigma, particularly among women and children
9. Evaluate strategies to strengthen health systems to support control of tuberculosis.
10. Increase understanding of the burden of disease, the modes of transmission and the impact of public health interventions for tuberculosis.

Conclusion: Tuberculosis is an infectious disease of poverty that accounts for a large burden of disease and mortality globally. Transformational research is required to drive discovery and to develop new tools. Once effective new tools are developed, it will be important to evaluate methods for their effective implementation and determine their impact on tuberculosis control. By identifying research priorities validated by key stakeholders, we hope that funders, researchers and policy-makers will adopt the priorities identified, and that this will lead to increased funding and transformational research. We envision that the outcomes will transform policies and practice that will accelerate progress towards achieving the Millennium Development Goals and the objectives of the Stop TB Partnership for eliminating tuberculosis as a global health threat by 2050.

1 Introduction

Tuberculosis (TB) remains an important global health problem. Although a number of important advances in its diagnosis and treatment have been made, further research is required to accelerate progress towards meeting the Millennium Development Goals (MDGs) and the Stop TB Partnership's goal of eliminating TB as a global health threat by 2050. TB is also a priority tropical disease for the Special Programme for Research and Training in Tropical Diseases (TDR), which has supported a broad range of TB research from diagnosis and treatment, new drug development, to social, economic and behaviour research in many developing countries over the past three decades.

As outlined in TDR's 10-year vision and strategy, Disease reference groups (DRGs) are crucial to TDR's stewardship mandate to acquire and analyse information on infectious diseases of poverty (1). TDR therefore commissioned the DRG for TB, leprosy and Buruli ulcer to provide an expert perspective on the current status of research on TB, identify critical research gaps and to prioritize areas for research. This report is specific to TB.

The TB Research Movement independently conducted a similar exercise to identify research priorities for TB using a different approach (2,3) and results were shared with the disease reference group. Reassuringly, there is substantial agreement and complementarity between the two respective sets of research priorities identified for TB, and the "International Roadmap for TB research" included priorities that were identified by the TDR process (3). The present report includes only the research priorities identified by the TDR process.

2 Methodology

2.1 Disease reference group

Technical experts from a representative range of countries were identified for the following thematic areas of TB research: epidemiology and control; health systems and operational research; case-finding and infection control; TB associated with infection with human immunodeficiency virus (HIV); vaccines, new drugs and diagnostics; and social science and gender. DRG members were asked to provide an expert perspective of their respective thematic areas and to identify research gaps and priorities. No formal systematic reviews were undertaken.

2.2 Identifying research priorities

A three-phase Delphi technique was used to prioritize the research gaps identified (Figure 2.1). In the first phase, at least 10 research priorities were identified for each thematic area and a justification for each research priority was provided. In the second phase the research priorities were compiled into a single list and distributed by e-mail to the DRG members. Each member of the DRG then selected 20 research priorities from the combined list, without providing justification for their selection. The 20 priorities from each member were then compiled into a single list, which was reviewed and

Fig. 2.1. Scheme showing the process of priority setting
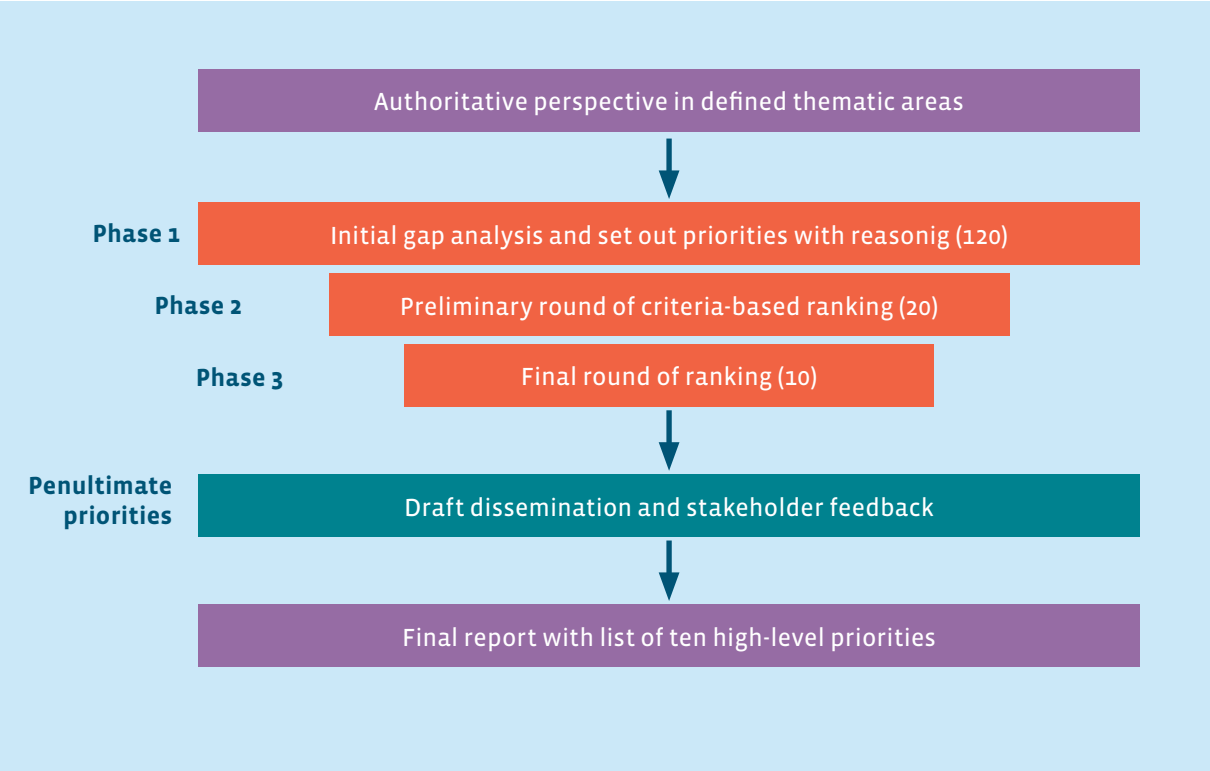
edited so that duplicate priorities were removed and similar priorities combined. In the third phase, a face-to-face meeting was held to identify a short-list of 10 high-level priorities for TB research. Prior to selecting the final priorities, the group agreed on the values that would be used to select the short-list (see Box 2.1). The final consolidated list of 10 priorities for TB was derived by consensus, and the justification provided for each priority selected.

2.3 Review

To ensure that the process for selecting the research priorities was transparent, fair and legitimate, regional and global stakeholders were invited to review and give comments on the research priorities. A regional consultation was held in the Philippines and the research priorities identified by the DRG were sent to all WHO regional offices for review and comment. All chapters in this report were reviewed by at least one internal and external reviewer.

2.4 Structure of the report

The thematic research areas are grouped under the following headings: epidemiology; developing new tools (vaccines, diagnostics and drugs); operational research, which includes health system research; social science and gender; and special focus areas (infection control, intensified case finding and HIV-associated TB).

Box 2.1. Values for selecting research priorities

- Curative versus preventive relevance

- Individual versus public health relevance

- Pro-poor versus pro-rich

- Related versus not related to Millennium Development Goals or other global targets

- Good versus poor feasibility (cost–benefit)

- Good versus poor generalizability

- Applying existing (old) versus developing new technologies

- Addresses versus does not address issues of equity, gender or social justice

3 Epidemiology

3.1 Burden of disease

Tuberculosis (TB) remains a major global health problem. Globally, in 2011 there were an estimated 630 000 cases (range: 460 000 – 790 000) of multi-drug resistant TB (MDR-TB) among the 12 million prevalent cases of TB; and an estimated 310 000 MDR-TB cases (range: 220 000 – 400 000) among notified patients with pulmonary TB *(4)*. The vast majority of TB cases occurred in three WHO regions: Western Pacific and South-East Asia Regions (59% combined) and African Region (26%) (Figure 3.1). Extensively drug- resistant TB (XDR-TB) had been reported from 84 countries by the end of 2011 *(4, 5)*. TB incidence has fallen globally for several years and declined at a rate of 2.2% between 2010 and 2011 *(4)*. Although the burden of prevalent TB is declining globally and in all WHO regions, it is nevertheless unlikely that the target of reducing prevalent TB by half by 2015 will be met, especially in the African and Eastern Mediterranean Regions *(6)*. The present rate of decline (2.2% per year) is insufficient to reach the target for the elimination of TB by 2050, defined as ≤ 1 TB case per million population per year *(4)*. In 2011, 1.4 million people died of TB; 0.99 million of which involved HIV-uninfected and 0.43 million, HIV-infected persons *(4)*. Worldwide, TB deaths have declined by just over a third since 1990. The target of reducing TB mortality by 50% of 1990 levels by 2015 has already been surpassed in the Region of the Americas and the Western Pacific Region, and may also have been reached in the Eastern Mediterranean Region. If the downward trend in mortality continues, the South-East Asia Region appears to be best placed to achieve the target among the three remaining regions *(4)*.

Fig. 3.1. Estimated tuberculosis (TB) incidences, 2011

Estimated new
TB cases (all forms)
per 100 000 population

- 0–24
- 25–49
- 50–149
- 150–299
- ≥ 300
- No estimate
- Not applicable
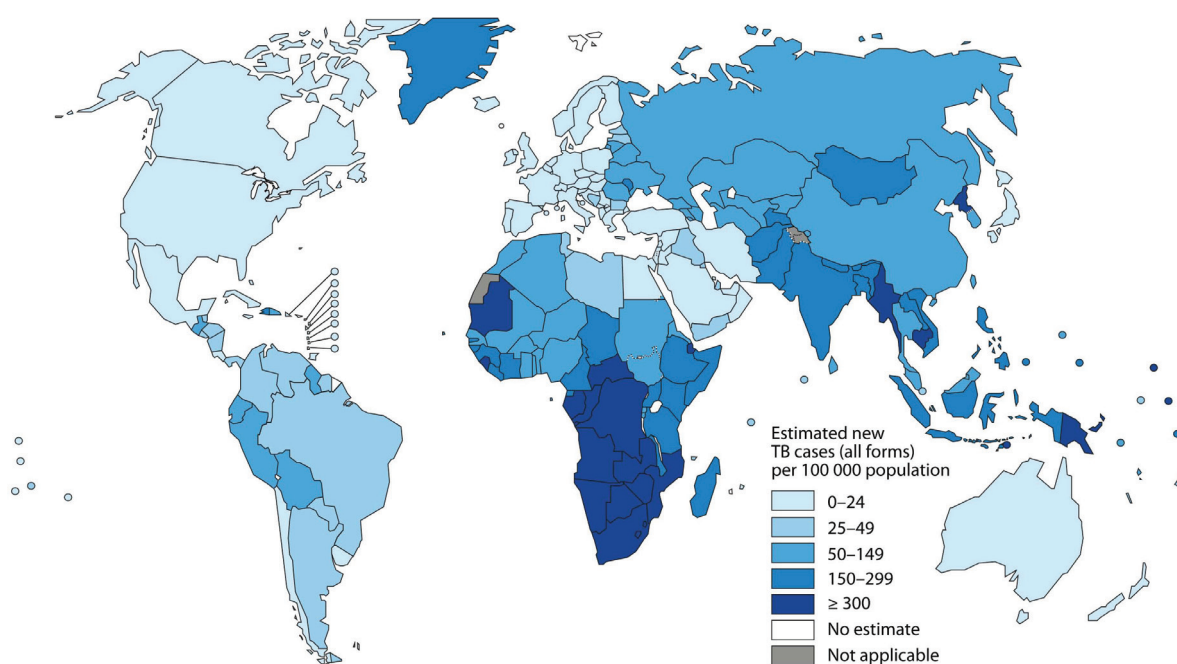
Source: WHO, 2012

3.2 Latent tuberculosis infection

It is estimated that one third of the world's population is infected with TB. The prevalence of TB infection increases with age and is thus relatively low in schoolchildren and young adults (7–9). Particularly high rates of infection have been found among the urban poor (10–13). In hyper-endemic settings, such as Cape Town, South Africa, 50% of adolescents and 88% of 31–35-year-olds are infected with TB (14, 15) and the annual risk of infection can be as high as 8% among adolescents. Latent tuberculosis infection (LTBI) among adolescents was associated with the following: being black or of mixed race, being male, being older, having a household TB contact, having a low educational level and a low income. Contacts of active cases have a high prevalence of TB infection if the index case is smear positive and if the contact is physically close (16–18). Mathematical modelling suggests that scaling up treatment of LTBI may have an important role to play in controlling TB (19).

3.3 Multi-drug resistant TB

MDR-TB is associated with increased mortality and longer treatment at much higher cost. The treatment outcomes for XDR-TB are even poorer, particularly among HIV-infected persons in resource-limited settings (20–22). Some, but not all, strains of MDR-TB may have reduced bacterial fitness (20, 21). A significant decrease in the prevalence of MDR-TB has been observed in some high- and middle-income countries (22–24), whereas eastern European and central Asian countries continue to represent hot spots for MDR-TB, with nearly one third of new and two thirds of previously treated TB cases affected by MDR-TB in some settings (4). A number of social and clinical epidemiologic factors are associated with MDR-TB. Surveys conducted in the USA have noted that the prevalence of drug resistance varies among different race/ethnic groups, age groups and by country of origin (25–27). In the USA and Europe, MDR-TB has been found to be associated with a history of previous TB treat-

ment, being male, being HIV seropositive, having a history of illicit drug and/or alcohol abuse, of having been incarcerated, and of homelessness (24, 28, 29). In India and the Republic of Korea, MDR-TB was associated with poor adherence to treatment, low socioeconomic status and low body mass index (30, 31) and, in Peru, with having a household contact with MDR- or XDR-TB (32).

3.4 HIV-associated TB

Being infected with HIV is strong risk factor for TB and has undermined TB control in HIV-prevalent countries as well as accounting for an increasing proportion of TB cases in some industrialized countries (33, 34). HIV-associated TB is generally less infectious and is associated with a shorter duration of disease (35, 36), which may limit the impact of its rising burden on TB transmission; however, repeated rounds of community-based intensified case-finding are required to reduce the prevalence of undiagnosed HIV-associated TB (37). HIV-infected TB patients have an increased risk of recurrence, largely due to reinfection in high-burden settings (38–42). They also have a high mortality risk (43–48), particularly those with MDR-TB and XDR-TB (49). TB is the commonest cause of death among HIV-infected individuals, including those on antiretroviral therapy (50).

Although HIV infection has been associated with institutional outbreaks of MDR-TB in both industrialized (51) and non-industrialized countries (51–53), it has not been shown to be associated with MDR-TB at a population level (54). However, by increasing the incidence of TB, HIV may be contributing to an increase in absolute numbers of MDR-TB cases.

3.5 TB in children

Globally, TB is a significant cause of morbidity and mortality among children. It is difficult to assess the worldwide extent of childhood morbidity from TB because of scarce and incomplete data, and because of the difficulty of diagnosing childhood TB with certainty in many countries. Reported disease rates are grossly underestimated and the prevalence of latent infection without disease is completely unknown in most areas of the world. The *WHO Global tuberculosis control report 2012* included the first ever estimates of the burden of TB disease among children, with best estimates of 490 000 cases and 64 000 deaths per year (*4*). In developing countries, childhood TB accounts for 15–40% of all cases and causes more than 10% of paediatric hospital admissions and deaths, particularly in countries with high HIV burdens (*55–59*). In contrast, in developed countries childhood TB represents 5% or less of all cases (*60*).

There is limited evidence that the burden of childhood TB in HIV-endemic regions has increased in recent years (*61*). Childhood TB is a common cause of pneumonia and death in some African countries, where cases of extrapulmonary TB in children are more frequently observed (*62, 63*). The incidence of childhood TB has declined in most industrialized countries (*64*). Childhood TB has a limited influence on TB epidemiology because children rarely infect contacts; however, the occurrence of TB in children is a marker for recent transmission and contributes to the pool of individuals with LTBI, from which future TB cases will arise. Programmes that target children for treatment of TB infection and disease may have little short-term effects on disease rates, but will be critical for long-term control of the disease.

Table 3.1 summarises the research priorities for TB epidemiological research.

Table 3.1. Research priorities for TB epidemiological research*

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Determine TB burden**	TB prevalence survey.	Identify disadvantaged groups with poor access to TB health care.	• Case detection suspected to be low in various settings • Need to understand the change in TB prevalence after long-term DOTS implementation in HBCs to quantify the impact of interventions. • Information needed to design strategies for improving access to TB health care of disadvantaged populations and to evaluate progress in meeting MDG targets.	TB prevalence in HBCs, stratified by gender, age, socio-economic status, residence status, children, prison, HIV status.
2	**Understand TB infection**	Prevalence of LTBI.	Identify risk groups for TB infection.	• Large fraction of global population has LTBI Without targeting this group, TB elimination impossible by 2050. • Quantify the impact of DOTS implementation on prevalence of TB infection.	Infection prevalence in HBCs, especially in children, and other high-risk subgroups.
3	**Identify correlates of protection**	Cohort studies.	Identification of targets for drugs and vaccines, diagnosis of those at high risk to develop TB as target for preventive therapy.	Large fraction of global population has LTBI Without targeting this group, TB elimination impossible by 2050.	Publications on candidate and validated targets.
4	**Identify social and biological drivers of drug-resistant TB**	• Determine fitness of strains with various drug-resistance-conferring mutations, eg through molecular epidemiological studies. • Comparison of settings with high and low rates of MDR-TB in TB patients without previous treatment history.	Identification of strains posing most important threat and identification of systems in need of reform to prevent MDR-TB and implementation of reform.	MDR- and XDR-TB are important threats that are inadequately understood.	• Publications on strain fitness. • Gene loci for resistance to second-line anti-TB drugs. • Systems identified for reform. • Success of reform demonstrated.

* For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
5	**Identify social and biological drivers of TB transmission in population**	• Molecular epidemiology using genotyping. • Epidemiological investigation for tracing transmission. • Spatial epidemiology using mapping.	• Determine the extent of transmission. • Identify dominant strains of MTB circulating in local settings. • Understand the interaction between pathogen, host, and the social determinants on MTB transmission.	• TB is transmitted in populations with low case detection. • The extent of TB transmission varied in populations even with high coverage of DOTS.	• Clusters of MTB identified in specific population. • Epidemiological traces of TB transmission.
6	**How best to reduce risk of TB transmission in health care and congregate settings**	• Risk assessment. • Implement. established methods. • Evaluate success and cost of these measures.	Case studies of successful infection control in high burden settings.	Infection control long neglected though known to be important.	Published case studies.
7	**Determine cost-effectiveness of various TB control measures**	Compare costs and effectiveness of various methods to prevent TB.	Policy guidance and utility for advocacy on cost-effective TB control measures.	Advocacy needed to maintain resources for TB control, resources need to be used in cost-effective way.	Published cost-effectiveness studies.
8	**Intervention studies to improve access with special emphasis on high HIV prevalence areas**	Implementation of interventions, evaluation through prevalence survey or molecular epidemiology.	Demonstrated effect of interventions for improved case-finding.	Case detection suspected to be low in various settings.	Published case studies.
9	**Project the trends of global TB incidence**	Modelling the trends of TB incidence in HBCs.	Estimate the incidence of TB in HBCs, and forecast the change of TB incidence.	To meet the MDG, reduce the incidence of TB.	TB incidence in low-, middle- and high-burden countries.
10	**TB research capacity strengthening in epidemio-logy, including field trials**	In part through MSc/PhD training, in part by participation in epidemiological studies and field trials.	Annual change of TB incidence in different settings.	Inadequate research capacity in HBCs.	Number of people trained at MSc and PhD level.

4 TB vaccines

4.1 New vaccines

4.1.1 Overview of future TB vaccination strategies

BCG, the only licensed vaccine against TB, has been administered to >90% of the world's children. BCG has shown consistent efficacy in protecting against childhood TB meningitis and miliary TB, but variable efficacy in protecting against adult pulmonary TB. Induction of T-cell immunity is thought to be important for control of *Mycobacterium tuberculosis* and this can be done most optimally by heterologous prime boost vaccination strategies.BCG is a prime vaccine and may in the future be replaced by a whole mycobacterium that is engineered to be less virulent, and/or to over-express the antigenic components of *M. tuberculosis*, which are thought to be important for protection. Attenuated *M. tuberculosis* engineered to avoid the immune escape mechanisms that this pathogen has evolved may also be used. Boost vaccines classically contain a few critical antigens, delivered within a live viral vector, or as a subunit together with a Th1-immunity-inducing adjuvant. The role of these vaccines would be to boost immunological memory induced by prime vaccination, increase the duration of immunity and improve the protection conferred by the priming event.

Ideally, the prime vaccine would ideally be given at birth, or soon thereafter, and the boost vaccine as part of the routine childhood Expanded Programme on Immunization vaccinations. This should prevent early TB disease and complement the known efficacy of BCG. The boost vaccine may be delivered at various times in a person's lifetime, most appropriately at the onset of adolescence, when the incidence of TB disease dramatically increases. As LTBI is common in settings where TB is prevalent, the ideal boost vaccine should also be able to prevent reactivation of disease. In addition, any novel vaccination strategy should be safe and effective in HIV-infected persons. Furthermore, therapeutic vaccines could complement chemotherapy by reducing treatment duration and increasing treatment efficacy, particularly in the context of antibiotic resistance or relative immune deficiency.

4.1.2 Efficacy of BCG

Some meta-analyses indicate that BCG's reported efficacy against infant TB in randomized controlled trials is 74% (95% CI: 62–83%) but only 52% (95% CI: 38–64%) in case–control studies (*65*). For infants, the reported protective effect of BCG against death from TB is 65% (95% CI: 12–86%), against TB meningitis, 64% (95% CI: 30–82%), against disseminated TB, 78% (95% CI: 58–88%) and against laboratory-confirmed TB, 83% (95% CI: 58–93%) (*65-67*). Another meta-analysis found that the protective effect against miliary or meningeal TB was 86% in randomized controlled trials (95% CI: 65–95%), and in case–control studies, 75% (95% CI: 61–84%) (*68*). Administering two doses of BCG does not confer added protection (*69, 70*).

The reasons for BCG's partial and variable efficacy may include exposure of vaccinees to environmental mycobacteria, which mask the effects of the vaccine. Distance from the equator is associated with higher BCG efficacy (*71*). Factors that vary with latitude include socioeconomic conditions, genetic background, climate, exposure to sunlight, diet and nutrition, environmental mycobacterial exposure, virulence of prevalent *M. tuberculosis* strains, storage and viability of BCG vaccine, and the quality of the BCG vaccine studies from which data were derived.

Finally, although protection induced by BCG may wane over time (*72*), it may persist for up to 10 years after infant vaccination (*66*). Many questions surrounding use of BCG remain unanswered. First, the strain-dependent determinants of its efficacy need to be delineated. Second, strategies for optimizing its efficacy for infants need to be determined, prior to assessing whether they could be implemented on a large scale. For example, delaying administration of BCG from birth to 10 weeks of age results in quantitatively and qualitatively "better" T-cell immunity at one year of age (*73*).

4.1.3 Safety of BCG

Disseminated BCG disease is known as "BCGosis" and is a relatively common complication in HIV-infected infants who have received BCG at birth (*74*). This may manifest either as a primary disease in the face of severe immune compromise or may emerge as an immune reconstitution inflammatory syndrome (IRIS) after antiretrovirals are started. Although data on the protective effect of BCG in HIV-infected children are lacking, the vaccine induces a very poor immune response in such infants (*75*). The revised guidelines of the WHO Global Advisory Committee on Vaccine Safety have therefore made infection with HIV a full contraindication to BCG vaccination (*68*). Ideally, BCG vaccination of infants born to HIV-infected mothers should be delayed until their HIV infection has been excluded with a viral amplification test; unfortunately, this strategy is difficult to implement in developing countries.

We await licensure of safer, whole mycobacterial prime vaccines. Until this happens, the use of BCG as a boost vaccine could be studied, i.e. following a vectored or subunit prime vaccination at birth, BCG would be given to HIV-uninfected infants.

4.2 New vaccines against TB

In order to have a real impact on TB's global burden, new vaccination strategies should significantly improve on BCG. This implies the need for vaccines or vaccine combinations that induce much more "optimal" immunity than BCG, not only in infants but also in adolescents and adults. Both pre- and post-exposure vaccines are needed.

In infancy, the optimal vaccine should fully prevent initial TB infection. To date, this has not been achieved, reflecting the exceptional resistance of extracellular mycobacteria to antibody-mediated or other bacteriolytic mechanisms. Soon after they infect an individual, mycobacteria hide and grow inside macrophages, but may be destroyed or their growth contained if the innate immune mechanisms or cell-mediated immunity is excellent. Most vaccination strategies aim to induce cell-mediated

immunity that may reduce the initial mycobacterial burden and ensure containment. However, virulent mycobacteria have evolved complex mechanisms to escape immune control.

It is believed that immunologically contained *M. tuberculosis* enters a stage of latency, characterized by altered metabolism and the expression of different genes from those expressed during the early stages of infection. A vaccine against latent TB should therefore target immunological mechanisms that may differ from those required to tackle the pathogen during early infection. Such targets may include antigens predominantly expressed during latency. Vaccines that specifically prevent reactivation should considerably reduce the risk of TB relapse.

Modelling studies suggest that effective TB vaccines are likely to have a greater impact on the TB epidemic than new diagnostics and drugs (*19*). Improved understanding of protective mechanisms against TB, either innate or adaptive, will directly contribute to the development of novel, better vaccines. In particular, immune escape mechanisms and factors associated with establishment or failure of latency need to be understood better. A summary of new TB vaccines in development can be found on the Stop TB website (www.stoptb.org/wg/new_vaccines).

4.2.1 Antigen discovery

Current candidate vaccines undergoing clinical trials contain classic mycobacterial antigens. Recent attention has focused on antigens expressed during LTBI, using simulations of the human state of latency with in-vivo or in-vitro models. The expression of these antigens in "dormant" mycobacteria reflects a distinct metabolic state (*76*). Antigens of the DosR regulon of *M. tuberculosis* are classic examples of latency-associated antigens, many of which are recognized by individuals with LTBI. However, recent data suggest that recognition of immunodominant antigens of *M. tuberculosis* expressed early after infection, such as ESAT-6, CFP-10

and antigen 85, may still be quantitatively greater in cases of LTBI (77). Nevertheless, the observation that recognition of the last-mentioned antigens is greater during latency than the acute phase of the disease suggests that targeting them during latency may be a strategy for immune control.

Although many latency-associated antigens are present in the genome of *Mycobacterium bovis* BCG, this vaccine does not induce immunity to them. Use of novel antigens will therefore have to involve BCG engineered to overexpress these antigens or enhance its primary delivery in boost vaccines. Such boost vaccines might be combined with early antigens of *M. tuberculosis* in a multi-phase approach.

Some promising antigens have recently been identified, including heparin-binding haemagglutinin (HBHA), an antigen that undergoes post-translation modifications that appear essential for immune recognition (78). These modifications consist of a complex methylation pattern in the C-terminal region, important for both B- and T-cell activation.

Non-peptidic antigens may also be involved in protection and are being investigated for their potential value as vaccine components. CD1-restricted lipid-specific T-lymphocytes are primed during infection with *M. tuberculosis*. Some of these lipids, e.g. diacylated sulfoglycolipids and glycerol mono-mycolate, appear promising as potential vaccines or vaccine components (79, 80).

The protective efficacies of current vaccines, which contain classic mycobacterial antigens, still need to be shown. Continued upstream research for the identification of other key antigens is therefore paramount. Our understanding of critical antigenic determinants remains incomplete.

4.2.2 Vaccine design

The portfolio of candidate TB vaccines, which reflects the present diversity of novel approaches, comprises live attenuated candidates (e.g. recombinant BCG or recombinant, attenuated *M. tuberculosis*), live vectored subunits (e.g. modified vaccinia

virus Ankara or adenovirus), recombinant proteins in adjuvants, DNA vaccines, and whole-cell killed mycobacteria.

Vaccine design is likely to be as critical for success as the choice of antigen. A recent analysis comparing immune responses to three novel TB vaccines with similar antigenic components administered to humans showed distinct patterns of T-cell activation (W. Hanekom, personal communication, 2012). This suggests that T-cell outcome is determined by viral vector or adjuvant interactions with the innate immune system. The importance of interaction with appropriate antigen-presenting cells has also been shown in a recent study of a subunit vaccine; the results indicate that, using appropriate formulations, immune cellular activation can be restricted to those dendritic cells that have captured the vaccine antigen. This may reduce the risks associated with broad non-specific stimulation of the immune system (81).

As vaccine design components are likely to be critical determinants of success, research is needed to delineate the optimal design for the desired immune responses. Furthermore, the route of delivery and its effects on the immune response have been inadequately studied, as have novel routes of vaccine delivery.

4.2.3 Preclinical studies

Although models exist for assessing the safety, immunogenicity and efficacy of new vaccines, the main challenge is whether the results can be transferred to humans. The current approach is to first screen candidates in murine challenge models, including immunodeficient mice, by aerosol challenge with virulent *M. tuberculosis*. These studies are often followed by those in guinea-pigs, whose inflammatory lung disease may better reflect that in humans. Ideally, all candidates should be studied in macaques, whose disease best reflects that of humans.

The limitations of murine and guinea-pig models are well-known, while those of the macaque model

include the small number of animals available worldwide for study, expense, and ethical issues. We therefore still do not have optimal models to test new TB vaccine candidates, and it is important to stimulate research into promising new models, such as the marmoset or miniature pig, which may be more sustainable than the macaque.

4.2.4 Assessment of safety and reactogenicity in humans

Clinical testing of new vaccines through phases I–III is tightly controlled by the regulatory authorities of individual countries or of groups of countries. Such authorities include the Food and Drug Administration (FDA) and the European Medicines Agency (EMEA), which do not necessarily share similar views, particularly for trials involving live recombinant, attenuated mycobacteria. Actual design of trials rests with the sponsor and local investigators, i.e. there is no uniform, standard approach.

Phase I and IIa clinical trials focus primarily on safety, although immunogenicity is usually also assessed. The first phase I trial involves healthy adults; it may also be used to find an optimal vaccine dose or doses. Phase IIa trials involve the target populations of a new vaccine. Should this target population be infants, some regulatory authorities insist on age de-escalation studies, proceeding from adults to adolescents to older children to younger children to toddlers, prior to infant studies. An ethical argument may be made against this approach, since some groups will be put at potential risk without standing to benefit from the future product.

Among concerns following new TB vaccine administration is the "Koch phenomenon", an excessive inflammatory reaction that arises when mycobacterial antigens are administered following previous mycobacterial exposure, such as BCG vaccination, TB infection or disease, or infection with non-tuberculous mycobacteria. Phase I trials therefore classically commence among populations who are naïve or near-naïve for mycobacterial exposure, prior to beginning phase IIa trials in persons known to be

latently infected with TB, and ultimately in those with TB disease. Given that HIV-infected individuals are at highest risk of developing TB, it is important that phase IIa trials should also be conducted in this population.

The lack of uniformity in clinical vaccine trial design and execution may ultimately compromise the development of new vaccines, as comparison of safety and reactogenicity of different vaccines is difficult. Similarly, uniformity among the requirements and rules of different regulatory authorities would be advantageous.

4.2.5 Assessing immunogenicity in human trials

Since no biomarkers of a protective immune response against TB are known, such responses induced by a novel vaccine should best be termed "vaccine take". Current assessment of vaccine take focuses on T-cell immunity, thought to be important for protection. Specific CD4 T-cells able to produce interferon-gamma (IFNγ) are critical for protection against TB. Therefore, specific IFNγ production by CD4 T-cells is measured in all current phase I or IIa trials of novel TB vaccines, either by an IFNγ ELISPOT assay or by an intracellular cytokine staining (ICS) assay. The IFNγ ELISPOT detects production of IFNγ by individual cells. Each cell producing this cytokine can be detected as a "spot" in a test well following incubation of peripheral blood mononuclear cells (PBMC) with specific vaccine antigens (*82*). Most spots probably originate from specific CD4 T-cells, although natural killer cells may contribute. In contrast, flow cytometric ICS assays detect IFNγ production specific to CD4 T-cells following incubation of whole blood or PBMC with specific antigens. Multi-parameter ICS assays allow assessment of complementary T-cell cytokine production, such as interleukin (IL)-2, tumour necrosis factor (TNF) and IL-17, within the same cells (*83*).

ELISPOT and ICS assays are classic examples of shorter-term assays, in which cells do not have time to proliferate, allowing a more quantitative assessment of the outcome. Many other assays may be

used to determine the T-cell response, as recently summarized in the WHO Panel Recommendations (*84*). Some assays have longer incubation periods (5–7 days), which may permit better assessment of cells that require longer periods for activation, such as central memory T-cells. The lymphocyte proliferation assay is a classic longer-term assay: PBMC are pre-stained with a dye such as carboxy-fluorescein succinimidyl ester (CFSE), incubated with specific antigens for 5–7 days, and proliferation of CD4 or CD8 T-cells measured by flow cytometry (*85*). Every time the cell replicates, it loses 50% of the original fluorescence intensity of the CFSE, allowing easy detection of expanded, specific cells.

Several variables determine assay success (*84*). For example, following blood collection, any delays in incubation or in isolating PBMC may compromise outcome. Measurements of immune response are more sensitive if freshly isolated PBMC is used rather than thawed PBMC after cryopreservation. Also, use of peptide, recombinant protein or whole bacteria as well as the dose of the antigen may have a significant impact on the assay results.

As mentioned above, the lack of standardization of immunological procedures across vaccine trials limits the use of the results for comparison of different vaccines. A good complementary strategy should involve cryopreservation of blood products for future measurement of outcomes ultimately shown to be surrogates of vaccination-induced protection against TB.

4.2.6   Biomarkers of protection

The exact nature of the immunological mechanisms that mediate protection at different stages of TB infection remains incompletely understood. T-cell mechanisms other than those described above may be important; for example, cytotoxic activity, regulatory activity or activity of non-traditional T-cells, such as γδ T-cells. Innate cells, including macrophages, natural killer cells, and dendritic cells, may also be critically important. The dogma that antibodies, complement, or other host components are non-critical players in immune control

may simply reflect our lack of understanding of the complexity of the response to mycobacteria. Nevertheless, evidence is emerging that a well-balanced T-cell response, including effector and regulatory arms, may be important for protection. Therefore, a quantitatively greater effector T-cell response following vaccination is not necessarily ideal.

For humans, modern immunological tools are unable to provide vaccination-induced correlates of protection against TB or correlates of risk of TB disease. Classic T-cell markers used to determine vaccine take, i.e. CD4 T-cell production of IFNγ, or even a polyfunctional CD4 T-cell response measured at 10 weeks of age following BCG vaccination of the newborn, do not correlate with protection, nor do proliferation responses. Use of unbiased screening methods, such as DNA microarray analysis of gene expression, may identify patterns that are associated with protection, and such approaches may ultimately permit discovery of novel correlates. Correlates of protection may only be validated in efficacy trials of new TB vaccines. Identification of biomarkers of protection against TB disease will immediately enhance TB vaccine development.

4.2.7 Assessment of efficacy in humans

Because of the huge cost of performing phase III trials to evaluate vaccine efficacy, proof-of-concept phase IIb trials may be planned to allow preliminary evaluation of efficacy (and assessment of extended safety/immunogenicity). The populations targeted for these trials need to have exceptionally high TB incidences (e.g. 2–5% per annum). The sample size is determined with a relatively low power to detect a difference between a vaccine and placebo; if no trend towards a vaccine effect is shown, progress to a phase III trial is unlikely.

The FDA accepts three approaches to showing vaccine efficacy: a clinical endpoint; an acceptable immune response endpoint, or the "Animal Rule", provided that certain criteria are met. Prospective, controlled, randomized clinical phase III trials demonstrating preventive efficacy for clinical endpoints, where the primary endpoint is the pre-

vention of disease, are believed to provide the most scientific rigour. Such trials are usually necessary in situations when the vaccine being considered is novel or is the first of its kind to be administered to the target population, and where there is no accepted immune response correlate of protection. Two efficacy trials are the norm, although one trial may be adequate if the result is compelling. The data must be robust and preferably generated in multiple sites.

Phase III trials of new TB vaccines will likely involve several thousands to tens of thousands of volunteers. Each such trial may cost over US$100 million to conduct, with enrolment probably taking at least a year to complete, and follow-up continuing for a minimum of 18–24 months. Such long follow-up periods are particularly relevant to trials involving infants and young children, among whom the peak incidence of TB disease is expected to occur between 12 and 24 months of age. These large trials will also be able to detect adverse low-frequency events (e.g. those of frequency <0.1%). Although much progress has been made in establishing new vaccine sites, the current complement will not be able to test more than 1–2 vaccines in phase III trials and many more sites are therefore needed.

The necessity of using clinical endpoints raises some issues, particularly for trials involving infants. TB disease is notoriously difficult to characterize in infancy and childhood, as the majority of cases are culture negative. The clinical signs and symptoms are non-specific, radiographic findings are difficult to interpret (particularly in early-stage disease, which is likely to be the case in a population under intense observation, as in a clinical trial) and tests of TB infection (tuberculin skin test (TST) and IFNγ release assays) are unreliable and subject to confounding. It has therefore been difficult to construct a robust case definition for use in such trials. Similar problems beset trials planned for HIV-infected adult populations. Definition of clinical endpoints for use in vaccine trials, particularly those involving infants and HIV-infected individuals, should therefore be a research priority.

4.3 TB immunotherapy

Advances in our understanding of the immunopathogenesis and host–pathogen interactions in TB have resulted in a range of techniques where immunotherapy has the potential to improve TB treatment. These include shortening the duration of antibiotic therapy, improving success rates for the treatment of MDR- and XDR-TB, and limiting the pathology to improve clinical outcome. Immunotherapeutic strategies can be divided into the following categories: 1) immunoregulatory approaches that seek to "realign" or improve the immune response by promoting protective (Th1) immunity or blocking immune-dampening (Th2) responses; 2) immunosuppressive therapy that aims (i) to improve TB chemotherapy by increasing drug penetration into the granuloma or enhancing TB responsiveness to antibiotic killing and/or (ii) to reduce immunopathogenesis and improve clinical outcome; and 3) supplementary effector cytokines that are intended to assist immune-mediated antibacterial activity. Candidate TB immunotherapies are at various stages of clinical development. Some approaches have been tested in animal models, while others have been evaluated in clinical trials (86).

Priorities for TB vaccine research are described in Table 4.1.

Table 4.1. Research priorities for TB vaccine research*

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Delineation of biomarkers of protection against TB disease**	Biomarker discovery in longitudinal studies of resistance against/risk of TB disease following natural MTB infection or in TB vaccine trials.	Blood test that can be used to predict whether new vaccines tested in phase I trials will be effective.	Currently, no reliable biomarkers of vaccination-induced protection exist As a result, large-scale expensive studies have to be completed to gain insight into efficacy.	• Initially, newly identified biomarkers, or combination of markers, through hypothesis-driven and hypothesis-generating approaches (training set), followed by validated markers (test set). • True validation only possible in phase IIb/III trial of an effective new vaccine.
2	**Description and understanding of most optimal vaccine antigen, and antigen delivery/ adjuvant design**	• Testing of immune recognition of MTB antigens, including proteins, peptides, lipids, lipopeptides and glycoproteins, in different stages of TB infection and disease. • In-vitro testing of human innate immune cell activation, and of T-cell induction, by vaccines delivered in various formulations, including recombinant bacteria, viral vectors, adjuvants, novel delivery systems – and describing mechanisms of interaction with innate immune system. • Combining antigens into new vaccine candidates. • Correlating immune cell activation with outcome in appropriate animal studies, and ultimately in human studies.	• Better understanding of immune recognition of multiple MTB antigens, including mechanisms of immune recognition. • Likely action: better understanding of the vaccine design that is likely to induce the most "optimal" immune responses.	• Our current repertoire and understanding of antigens and their potential role in protection, remain limited. • Our understanding of recognition of non-classical antigens and of the role of non-immunodominant proteins is limited. • Antigen composition of vaccines is important; however, the interaction with the innate immune system is the critical determinant of vaccine take and pattern of immune activation.	• New antigens that may be tested as candidates for vaccines. • Human innate immune cell activation and human T and other cellular activation, both in vitro and in vivo. • New vaccines.

* For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
3	**Development of new animal models to study TB vaccines**	Development and testing of different primate species, including the marmoset, or models of clinical settings, such as latency or immune deficiency.	New, affordable animal models that better reflect humans.	There are many limitations to current models, eg mouse models may not reflect human disease; non-human primates which do seem to reflect human disease quite well are very expensive.	Number of new models developed.
4	**Standardization and harmonization of preclinical testing of new vaccines**	Review of current approaches and delineation of one approach to efficacy and immunogenicity testing that can be used for all vaccines of a certain type, or to model a certain clinical setting.	A template for pre-clinical design of new vaccine candidates.	• Very diverse approaches to new TB vaccine candidates reported in the literature. • Comparisons therefore difficult.	Templates for preclinical testing of a recombinant whole bacterial vaccine, of a viral vectored vaccine, etc, to simulate various clinical situations, eg MTB-infected, HIV-infected, IRIS, etc.
5	**Standardization and harmonization of phase I and IIa trial clinical procedures**	Review of current approaches to design, and if necessary, small studies to "validate" best approaches to study safety in various populations, including healthy adults, adolescents and infants, TB-infected persons, HIV-infected persons.	A template for clinical design of phase I/IIa trials of different new vaccine candidates.	While diversity of vaccines makes comparison of different vaccine candidates in clinical trials difficult, the current ad lib approach to vaccine trial design makes this even more difficult. There is limited scope for phase IIb/III testing worldwide but deciding which candidates should move ahead may be difficult without comparisons.	Templates for phase I/IIa design of a recombinant whole bacterial vaccine and of a viral vectored vaccine, etc, in different populations, eg, MTB-infected, HIV-infected, etc.
6	**Standardization and harmonization of phase I/IIa trial immunogenicity procedures**	• Review of best approaches currently available for certain vaccine candidates, for specific phases of trials, and in specific vaccine target populations. • Qualification and/or validation of best approaches.	Validated assays for testing vaccine take in different clinical trials.	As for clinical procedures, comparison of different vaccines is the objective, prior to testing in phase IIb or III trials.	SOPs for phase I and IIa vaccine use the design of a recombinant whole bacterial vaccine, of a viral vectored vaccine, etc, in different populations, eg, MTB-infected, HIV-infected, etc.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
7	**Development capacity for phase III testing of new TB vaccines**	• Defining criteria and assessing appropriateness for phase III TB vaccine site development, in populations from diverse genetic backgrounds. • Preparing sites with mock phase III trials in infants and adolescents, which may define local epidemiology optimally.	Multiple, multi-continent sites that can be used for phase III testing of new vaccines.	Current capacity exists to test perhaps two vaccines in phase III trials in infancy, and one vaccine in phase III trial in adolescence.	Number of new sites with capacity to test new vaccines in phase III trials of infants and of adolescents.
8	**Definition of clinical endpoints of phase IIb/III trials**	Research to better diagnose TB in infants/children and in HIV-infected persons, in particular (compare diagnosis priorities).	Optimal diagnostic tests to reliably diagnose TB in infants/children and in HIV-infected persons.	Hard diagnostic endpoints, eg a positive smear or culture, are available in a minority of infants and HIV-infected persons with TB, making outcome determination in phase III trials virtually impossible.	Number of new, validated tests to diagnose TB in infants/children and in HIV-infected persons.
9	**Determine the best use of the current TB vaccine – BCG – for optimal efficacy**	• Phase IV trials of different strains of BCG, of dose, of routes of administration, and of age of administration. • If clinical trials not possible or too expensive, examine variables with the "best" immunological endpoints.	Optimal manner to use BCG to protect against lung TB in childhood, and to improve protection against TB meningitis and miliary TB, as well as to best induce immunity that may be enhanced by boost vaccines.	BCG has never undergone the rigorous testing common for current childhood vaccines – many variables surrounding its use remain unknown. It is unlikely that a replacement for BCG will be marketed in the next 10 years. There is little point in testing multiple boost vaccine candidates if we cannot get the prime right.	• Optimal strain, dose, route and age of administration of BCG for optimal protective efficacy in children known. • Underlying mechanisms for observations described.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
10	**Develop new strategies for TB vaccination of HIV-exposed infants**	• Testing the effect of recent introduction of universal early ART in HIV-infected infants on epidemiology and presentation of BCGosis (such a study may no longer be possible, however). • Phase IIa testing of whether novel viral vectored or subunit/adjuvant vaccines may be used as safe prime vaccines at birth, followed by a BCG boost when shown not to be HIV-infected. • Phase IIa testing of safer prime vaccines, eg recombinant strains of BCG and or MTB; ultimately phase IIb/III testing in HIV-exposed infants.	A safe, effective vaccine regimen for HIV-exposed infants.	HIV infection in infancy is currently associated with BCGosis, which is common and which may cause much morbidity BCGosis may manifest due to severe immune deficiency or as an IRIS syndrome. Safer whole bacterial vaccines are still many years from licensure – safer alternate options are needed now HIV-exposed infants are often from households at high risk of TB disease.	• Safety and immunogenicity of novel vaccination strategies in HIV-infected infants • ultimately efficacy of new strategy in HIV-exposed infants.

5 New Diagnostics

5.1 Overview

Lack of rapid and accurate diagnosis and case detection are major obstacles to TB control. TB diagnosis for the most part relies on antiquated tools such as direct smear microscopy, solid culture, chest radiography and the tuberculin skin test (TST). The limitations of the existing diagnostics toolbox have been exposed by the HIV epidemic (*87, 88*) and by the emergence of MDR-TB and XDR-TB. Diagnostic delays and health system failures often result in missed or late diagnoses with serious consequences for TB patients (*89*).

In the past few years, unprecedented interest and activity have been focused on the development of new tools for TB diagnosis (*90–92*). Primary diagnostic trials are carried out to generate data on test accuracy and operational performance; however, systematic reviews and meta-analyses provide the best synthesis of current evidence on any given diagnostic test. Although several systematic reviews are now available, much of the evidence base is focused on test accuracy (i.e. sensitivity and specificity); accuracy studies on TB diagnostics are often poorly conducted and reported (*93*). There are limited data on outcomes such as the accuracy of diagnostic algorithms (rather than of single tests), the relative contribution of such algorithms to the health-care system, the incremental value of new tests, the impact of new tests on clinical decision-making and therapeutic choices, as well as their cost-effectiveness in routine programme settings and impact on patient-important outcomes (*94*). In turn, this poses problems for policy and guideline development, because test accuracy is a surrogate for patient-important outcomes (*95*). The new diagnostics pipeline for TB is rapidly expanding (*87, 96*) and details are available from: http://apps.who.int/tdr/svc/publications/non-tdr-publications/diagnostic-tool-tb

5.2 Optimized smear microscopy

Direct sputum smear microscopy remains the primary means for diagnosing TB in most resource-limited countries. Because of the limitations of such microscopy, considerable effort has been expended to identify methods that can optimize its yield and accuracy (*97–100*). These include fluorescence light-emitting diode (LED) microscopy , use of sputum-processing methods, and optimization of specimen collection for same-day diagnosis (i.e. front-loaded microscopy) (*101*). There is considerable evidence that LED technologies are more sensitive than Ziehl–Neelsen (ZN) microscopy and that LED microscopy requires less time to read slides than ZN microscopy (*102*). In view of the evidence, WHO recommended in July 2010 that conventional fluorescence microscopy be replaced by LED microscopy in all settings and that it be phased in as an alternative to conventional ZN microscopy in both high- and low-volume laboratories. WHO also endorsed use of same-day smear diagnosis. Combining LED microscopy with same-day diagnosis is another interesting approach to improving case detection (*103*). Mobile-phone-based microscopy (*104*) and automated detection systems using image processing (*105*) are other novel approaches that have been proposed, although their use has yet to be adequately validated.

5.3 Improved and newer culture methods

5.3.1 Automated liquid cultures

Automated liquid culture systems such as BacT/ALERT® MP (bioMérieux Inc, Durham, NC, USA) and BD BACTECTM MGITTM (BD, Sparks, MD, USA) are currently considered the gold standards for isolating mycobacteria. Meta-analyses have shown that liquid systems are more sensitive for detecting mycobacteria and may increase the case yield by 10% over solid media (*106, 107*). Such systems also reduce the delays in obtaining results from weeks to days. Use of liquid media for drug susceptibility testing (DST) produces even greater time savings. However, liquid systems are prone to contamination and require stringent quality assurance and training standards. In 2007, WHO released a policy statement on their use and on species confirmation through rapid antigen detection tests (*108*).

5.3.2 Unconventional and novel culture methods

Unconventional and novel culture-based approaches for TB diagnosis and drug resistance testing include microscopic observation DST (MODS) (*26*), thin-layer agar (TLA) (*109*), and direct nitrate reductase assay (NRA) (*110*). Recent reviews have summarized the characteristics and potential role of these approaches (*111–113*). Although such methods are promising, since they permit use of inexpensive materials and give turn-around times similar to those of liquid culture, they are not well standardized and require extensive training and optimization prior to routine clinical use. In 2009, a WHO Expert Group recommended that selected non-commercial culture and DST methods (MODS, NRA and colorimetric redox indicator assays) be used as an interim solution in resource-constrained settings, in reference laboratories or those with sufficient culture capacity, while capacity for genotypic and/or automated liquid culture and DST is being developed (*114*).

5.3.3 Molecular tests

Nucleic acid amplification tests (NAATs) have been used for many years, although mainly only in high-income countries (*115*). Current NAATs have high specificity, but modest and variable sensitivity, especially for smear-negative and extrapulmonary TB (*116–119*). Several newer NAATs have been developed recently, including loop-mediated isothermal amplification (LAMP) (Eiken Chemical Co. Ltd., Tokyo, Japan) — a simplified manual NAAT designed for peripheral laboratory facilities (*120*), —and the Xpert® MTB/RIF assay (Cepheid, Sunnyvale, California, USA) — a fully automated NAAT platform that can detect TB as well as rifampin resistance. Both of these tests are intended to replace or supplement microscopy at health centres and district hospitals. Xpert® MTB/RIF has a sensitivity of 98.2% for smear-positive TB, 72.5% for smear-negative TB and a specificity of 99.2 % when used on three specimens. The assay also correctly identified 97.6% of patients with rifampicin resistance (121). Xpert® MTB/RIF performed similarly under field conditions and has reasonable sensitivity with non-sputum specimens, making it potentially useful in the diagnosis of extrapulmonary TB (*121, 122*).

Following the WHO policy of December 2010 that endorsed the roll-out of the Xpert® MTB/RIF assay, much progress has been made in reducing its cost (*123*). In June 2012, UNITAID, the United States Agency for International Development (USAID), the United States President's Emergency Plan for AIDS Relief (PEPFAR) and the Bill & Melinda Gates Foundation announced an agreement with Cepheid to reduce the cost of the test to US$ 9.98 per cartridge. This price is applicable to over 145 purchasers in low- and middle-income countries. As part of the agreement, UITAID committed up to US$ 30 million to achieve the cost reduction and to scale up access through an accelerated roll-out of the assay via the TBXpert Project, which made available up to US$ 25.9 million to 21 recipient countries for this purpose (http://www.who.int/tb/laboratory/mtbrifrollout/en/index.html). As of September

2012, a total of 898 GeneXpert® instruments and 1 482 550 Xpert®MTB/RIF cartridges had been procured for the public sector in 73 HBCs, with South Africa accounting for the majority.

The evidence base on Xpert® MTB/RIF has also grown rapidly with a large number of published studies on test accuracy, and a growing number of implementation studies in HBCs. In 2013 WHO is planning to update its 2010 TB diagnosis policy; it is expected that the new policy will provide guidance on the use of Xpert® MTB/RIF for extrapulmonary and childhood TB.

Line probe assays (LPAs) have been rolled out in many countries for molecular detection of drug resistance in smear-positive specimens. Two commercial LPAs are available, the INNO-LiPA-Rif.TB® (Innogenetics NV, Gent, Belgium) and GenoType® MTBDRplus (Hain Lifescience GmbH, Nehren, Germany). Meta-analyses have shown that LPAs are highly accurate, and the GenoType assay, in particular, performs exceedingly well for rapid detection of rifampin resistance in smear-positive sputum specimens (124, 125). In 2009, Hain Lifescience GmbH introduced a newer assay (GenoType® MTBDRsl) (126) which permits simultaneous detection of the *M. tuberculosis* complex and resistance to fluoroquinolones and/or aminoglycosides/cyclic peptides and/or ethambutol from smear-positive pulmonary specimens or culture isolates. Use of a combination of GenoType® MTBDRplus and GenoType® MTB-DRsl therefore permits rapid detection of XDR-TB. Because LPAs currently require routine specimen processing, DNA extraction and conventional polymerase chain reaction (PCR) in a multi-room facility, their use is limited to reference laboratories. In 2012 a WHO Expert Group reviewed the evidence on GenoType® MTBDRsl and found that, although its specificity for detecting resistance to fluoroquinolones and second-line drugs was high, its sensitivity was suboptimal (127). Therefore, while GenoType® MTBDRsl has the potential to be used as a rule-in test for XDR-TB where capacity to use LPAs is available, it cannot be used as a substitute for conventional phenotypic drug-susceptibility testing.

5.4 Immune-based tests

5.4.1 Serological, antibody detection tests

Current commercial serological tests are of little clinical value because of their suboptimal accuracy and highly inconsistent results (128–130). In 2011, WHO published a strongly negative policy recommendation against the use of such tests for TB diagnosis (131). However, use of combinations of selected antigens may provide higher sensitivities than single antigens (132).

5.4.2 Antigen detection tests

Antigen detection has the potential to overcome some of the well-recognized problems with antibody detection assays, especially in HIV-infected populations. Urinary lipoarabinomannan (LAM) detection was considered a particularly good candidate, based on early studies, especially in HIV-infected persons (133). Unfortunately, field studies in high-burden settings have shown LAM performance to be variable and suboptimal, with lower sensitivity than expected (134, 135). However, some recent data suggest that LAM may perform better in HIV-infected individuals with advanced immunosuppression, in whom smear microscopy often tends to be negative (136, 137).

5.4.3 Interferon-gamma release assays

Until recently, diagnosis of latent TB infection (LTBI) depended solely on TST, a test with several limitations (138). A major recent advance has been the development of T-cell-based IFNγ release assays (IGRAs). Two such assays are currently available as commercial kits: the QuantiFERON®-TB Gold In-Tube (QFT-IT) assay (Cellestis Ltd., Carnegie, Australia) and the T-SPOT.TB® assay (Oxford Immunotec, Abingdon, England).

Systematic reviews have reported strong evidence that IGRAs have very high specificity that is unaffected by BCG vaccination (139, 140). The TST, in contrast, has high specificity for non-BCG-vaccinated populations but only modest and inconsistent

specificity for BCG-vaccinated populations. In low-incidence settings, IGRA results correlate well with surrogates of TB exposure. The high specificity of IGRAs is proving useful in BCG-vaccinated individuals, particularly in countries where TST specificity is compromised by BCG vaccination after infancy or by multiple BCG vaccinations (*140*).

The sensitivity of IGRAs and TST is not consistent across tests and populations, but IGRAs appear to be at least as sensitive as TST (*140*). Diagnosis of active TB depends on microbiological detection of *M. tuberculosis* (*141,142*). Immune-based tests do not directly detect *M. tuberculosis*; instead they indicate a cellular immune response to it. Because IGRAs cannot distinguish between latent and active TB, a positive IGRA result may not necessarily indicate active TB. Furthermore, a negative IGRA result would not conclusively rule out active disease in an individual suspected to have TB (similar to the results of a TST).

Immunosuppression due to HIV has an impact on the results of TST as well as of IGRAs. The sensitivity of both QFT-IT and T-SPOT.TB® appears to be lower in HIV-infected TB patients than that of the published estimates for HIV-uninfected patients (*143*). Also, there is a clear association between increasing degrees of immunosuppression and higher rates of indeterminate IGRA results. IGRAs should therefore not be used as rule-out tests for active TB in HIV-infected persons, especially in high-burden settings.

Use of IGRAs is steadily increasing in low- and intermediate-burden countries (*144*). Three main approaches have been recommended for the use of IGRAs: TST should be replaced by IGRA; either TST or IGRA may be used; or a two-step approach — first TST followed by IGRA. There is considerable diversity in how countries currently recommend and use IGRAs. The two-step approach seems to be the most dominant strategy, but this may partly be due to cost considerations.

Evidence on the prognostic value of IGRAs is still limited (*140, 145*). None of the existing tests for LTBI

can easily identify the subgroup of patients that is at highest risk of progressing to disease (*146*). A majority of those positive by TST or IGRAs will not progress to TB, and these individuals presumably do not need preventive therapy. This may, in part, be because IFNγ alone might not be sufficient as a biomarker for disease progression or because a single, one-time IGRA result might not be adequate to predict those at risk (*146*). There is growing evidence that the performance of IGRAs is different in high and low TB incidence countries (*147*). The role, if any, of such assays appears to be rather limited in low-income, high-burden countries. In 2011, WHO recommended that, given their comparable performance but increased cost, IGRAs should not replace the TST as a public health intervention in resource-constrained settings in low- and middle-income countries (*148*).

5.4.4 Improved skin tests

A well-recognized limitation of the conventional TST is the lack of specificity of the purified protein derivative (PPD) that is uses. Attempts have been made to replace PPD with antigens (such as ESAT-6) that are specific to *M. tuberculosis*. Small-scale, phase I trials of this improved skin test have shown promise, but further validation is needed (*18, 149*).

5.5 Point-of-care technologies

The ideal TB diagnostic test would be a simple, low-tech, point-of-care (POC) test that can be rapidly performed and yields accurate results (*150*). Currently, no test meets all of these requirements. However, several agencies and groups are developing POC tests for TB, including novel serological assays, detection of volatile organic compounds in breath, hand-held molecular devices, microchip technologies, and tests that exploit approaches such as microfluidics, nanotechnology, proteomics and metabolomics.

5.6 Diagnostics for childhood TB

Diagnosing childhood TB is a challenge and although many new TB diagnostics are in the pipeline, few have been evaluated extensively in children (*151,152*). Despite the lack of strong supportive evidence, many guidelines on IGRAs have suggested that they could be used as an adjunct tool for diagnosing TB in children; (*153–155*) however, their use should not be a substitute for appropriate specimen collection for microbiologic diagnosis (*153*). All new diagnostics should be validated among children, especially young. children and HIV-infected children.

5.7 Diagnostics for smear-negative TB

Smear-negative TB continues to pose challenges, especially in areas with high HIV prevalence. Improved NAATs such as Xpert® MTB/RIF appear to be promising in this regard (*156, 157*).

5.8 Cost-effectiveness and potential impact of new tools

For resource-poor HBCs important concerns are the cost of introducing new tools, their successful implementation and their long-term sustainability. Unfortunately, only a few studies have examined cost-effectiveness, while even fewer have modelled the potential impact of the introduction of new diagnostic tests. Development of a standardized methodology for costing TB diagnostic tests would enable improved and more generalizable costing analyses. This would then provide a strong foundation for more advanced analyses that evaluate the full economic and epidemiological impact resulting from the implementation of validated new diagnostics (158). Modelling studies suggest that novel diagnostic tests have the potential to be cost-effective tools in TB control if they have high specificity and sensitivity (for cases missed by existing diagnostic tests) and are also affordable (*159*).

Additional evidence from implementation studies should be generated to support the introduction of new diagnostics. Downstream impact assessment is necessary, especially after a new diagnostic is introduced into a TB control programme. Operational research is also essential to improve service delivery, to understand why programmes fail, and to guide optimal implementation of new tools.

The research priorities discussed in this chapter are summarized in Table 5.1.

Table 5.1. Research priorities for new TB diagnostics *, **

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Development of a rapid, accurate POC test for active pulmonary TB**	Biomarker discovery, followed by incorporation in a highly sensitive POC platform and then clinical validation.	A POC test for active pulmonary TB that will meet the user-defined specifications.	Currently, there is no POC test for TB that can be used at the health clinic level – diagnostic delays, therefore, are common.	• Number of fully validated POC tests for detection of active TB that are more sensitive, simpler and as affordable as smear microscopy. • Proportion of eligible cases diagnosed for active TB using POC tests that are more sensitive, simpler and as affordable as smear microscopy.
2	**Development and validation of tools for rapid detection of drug resistance, including for XDR-TB, and standardization of DST for second-line drugs**	• Identification and characterization of mutations associated with second-line drug resistance. • development of newer generation molecular assays for MDR and XDR-TB; improved standardization of existing tests for second-line DST.	Rapid molecular (genotypic) assays for MDR-/XDR-TB that will allow rapid identification of drug-resistant TB.	• Although LPAs are highly accurate for rifampin resistance, accuracy is lower for izoniazid and other drugs. • Second -line DST continues to be a challenge Mutations are not well defined and standardization is a problem with phenotypic methods.	• Number of diag-nostic markers of drug resistance identified for second-line drugs. • Proportion of eligible cases detected for drug resistance (MDR and XDR) with accuracy similar to culture but capable of provid-ing results in a few hours (or days) instead of weeks.
3	**Intensified, active case detection strategies for early detection of active TB in HIV-infected persons (at the clinic level and in the community)**	Development and validation of an algorithm (including new tests) for rapid detection of TB in HIV-infected persons.	A validated algorithm that will enable detection of TB in a large proportion of HIV-infected persons with TB disease.	• Passive case detection methods do not work well in areas with high HIV prevalence Undiagnosed TB is frequent in HIV-infected persons and can cause enormous morbidity and mortality. • Aggressive case detection approaches are needed to enhance case detection, reduce mortality and reduce transmission.	Proportion of HIV-infected persons with TB disease that are successfully detected by active case detection strategies.

*   For an explanation of the abbreviations and acronyms, see p. 8-9

** Adapted from: Pai M, et al.. New and improved tuberculosis diagnostics: evidence, policy, practice and impact. *Current Opinion in Pulmonary Medicine*, 2010 May, 16(3):271–84.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
4	**Improving current diagnostic algorithms to shorten the time required for establishing a diagnosis of smear-negative pulmonary TB and extra-pulmonary TB in HIV-infected persons and children**	Development and validation of an algorithm (including new tests) for rapid detection of smear-negative and extrapulmonary TB in HIV-infected persons and children.	A validated algorithm that will rapidly enable detection of smear-negative and extrapulmonary TB in a large proportion of HIV-infected persons and children.	Smear-negative TB, extrapulmonary TB and childhood TB are diagnostic challenges and available tests perform poorly in these cases of paucibacillary TB. Newer algorithms and tests are needed to get around the limitations of current methods.	Proportion of HIV-infected persons with smear-negative TB disease that are successfully detected by a new test or algorithm.
5	**Development of a test or algorithm that can accurately rule out active TB disease in HIV-infected persons to allow initiation of preventive therapy**	Development and validation of an algorithm (including new tests) for rapidly ruling out active TB (including smear-negative and extrapulmonary TB) in HIV-infected persons.	A validated algorithm that will enable exclusion of TB in a large proportion of HIV-infected persons prior to IPT.	In HIV-infected persons undiagnosed active TB is common. Before initiation of IPT, active TB must be ruled out. However, there is no easy-to-use and accurate method to achieve this in HBCs.	Proportion of HIV-infected persons initiated on IPT after ruling out active TB with a new test or validated algorithm.
6	**What biomarkers or combinations of markers will help distinguish the various stages of the spectrum of latent TB infection (from sterilizing immunity to subclinical active disease) and will allow accurate prediction of the subgroup of latently infected individuals that are at highest risk of progression to disease**	Biomarker discovery, followed by validation in clinical and longitudinal (cohort) studies for markers that can predict risk of progression to active TB.	Identification of a biomarker or combination of biomarkers that will allow accurate prediction of the subgroup of latently infected individuals that are at highest risk of progression to disease.	Existing tests for LTBI (TST and IGRAs) cannot distinguish the various phases of the latent TB spectrum. This means existing tests cannot be used to target IPT in the subgroup most likely to benefit from treatment. This results in over-treatment of a large number of latently infected persons.	• Number of diagnostic markers of risk of progression to disease identified. • Proportion of eligible cases screened for prediction of future progression of latent TB infection to active disease, in both HIV-infected and uninfected subjects.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
7	**Development of a rapid test for childhood TB that will not depend on sputum specimen testing**	Development and validation of a test or an algorithm (including new tests) for rapid detection of TB in children, without requiring sputum specimens.	A test (preferably POC) that can use non-sputum specimens (eg urine, breath condensate or saliva) for rapid detection of TB in children.	Childhood TB is a diagnostic challenge and available tests perform poorly in these cases of paucibacillary TB. Also, since young children are unable to produce sputum, it will be helpful to use alternative specimens such as urine, saliva or breath condensate.	• Number of fully validated POC tests for detection of active TB in children that are based on non-sputum specimens. • Proportion of eligible childhood TB cases diagnosed for active TB using POC tests that are based on non-sputum specimens.
8	**Define different ways of operationalizing and implementing existing policies on HIV testing of TB patients and TB screening of HIV-infected persons**	Operational research on different ways of operationalizing and implementing existing policies on HIV testing of TB patients and TB screening of HIV-infected persons.	Identification of at least one feasible approach that might work best and therefore can be scaled up.	• Existing policies on HIV testing of TB patients and TB screening of HIV-infected persons are poorly implemented. • A large proportion of TB patients are not tested for HIV, and HIV-infected persons are not screened for TB This results in undiagnosed co-infection morbidity/ mortality, and continued transmission in the community.	• Proportion of HIV-infected persons screened for active and latent TB. • Proportion of active TB patients screened for HIV infection.
9	**Once new diagnostics are approved and available, what factors can enhance their actual delivery and implementation at the programmatic level in HBCs?**	Operational research on different ways of implementing new diagnostics in national TB programmes in high-burden settings.	Identification of at least one feasible implementation approach that might work best and therefore can be scaled up.	• Availability of new tools does not necessarily ensure their adoption and implementation. • Translation of policy into practice requires better understanding of barriers to implementation and methods to overcome such barriers.	• Number of new diagnostic tools included in national TB programmes. • Number of operational research studies done on delivery and implementation in programme settings.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
10	**What is the likely epidemiological impact of widespread LTBI diagnosis and treatment in HBCs, and what contribution will LTBI diagnosis and treatment make towards the attainment of the Stop TB Partnership's target for TB elimination?**	Mathematical modelling study.	Modelling study will inform the debate on when to begin to focus attention on LTBI diagnosis and treatment in HBCs.	LTBI diagnosis and treatment are currently not priorities in high-burden countries. However, as TB incidence falls, they can become priorities. Also, some recent modelling studies suggest that TB elimination will require strategies aimed at LTBI management.	Global incidence of TB.

6 New drugs for treatment of TB

6.1 Overview

If prescribed and taken appropriately, the drugs currently used for the treatment of TB are efficacious in the vast majority of drug-sensitive (DS) cases. However, these drugs have lengthy and complex regimens that are difficult for patients to adhere to once they feel better symptomatically (typically after only a few weeks). Why it requires such long treatment times to cure TB is not fully understood but may be related to the ability of a sub-population of *M. tuberculosis* within a host to enter a non-replicating, sporadically replicating, or slowly replicating state that is impervious to most current drugs. For example, isoniazid administered alone at the standard dose kills over 90% of the infecting mycobacteria in the first two days of treatment (*160*). Nevertheless, isoniazid-containing combination therapy must be taken for months to eradicate the relatively few remaining persistent bacteria and ensure that patients will not relapse once therapy is stopped. This phenomenon is variously referred to as "phenotypic resistance", "tolerance", or, most commonly, "persistence". The difficulty in killing low infecting numbers of *M. tuberculosis* organisms is highlighted in the treatment of LTBI. The relationship, if any, between persistent bacilli in active TB and latent organisms in LTBI is not understood, although both conditions require lengthy periods of therapy despite genotypic sensitivity to the treating agents. Substantive progress towards eliminating TB as a public health problem will require elimination of the reservoir of latent TB infections, which are estimated to be present in approximately one third of the world's population. Furthermore, there are no current methods to accurately estimate the percentage of LTBI caused by MDR- and XDR-strains of *M. tuberculosis*; however, drug-resistant (DR)-LTBI must be increasing with the incidence of DR-TB. Eradicating these resistant infections poses an even more difficult challenge than eradicating DS-LTBI.

Current first-line TB drugs, especially the rifamycins, are also limited by drug–drug interactions (DDIs), which make them difficult to co-administer with antiretroviral agents, especially protease inhibitors (PIs). This arises because rifamycins interact with key cytochrome P (CYP) 450 enzymes (*161*) which makes appropriate treatment of TB patients who are HIV-co-infected complicated, especially in high burden settings. Second-line drugs used for treating MDR- and XDR-TB are much less efficacious, often highly toxic, and considerably more expensive than first-line drugs. According to the latest WHO estimates, only 19% of the world's notified MDR-TB patients are receiving appropriate treatment (*4*). Current recommended treatment regimens are not easily amenable to widespread scale-up.

Historically, paediatric TB has been largely ignored in control and drug research and development (R&D) (*162*). Recently, however, key issues in paediatric TB treatment have received more attention and a number of current sponsors of new TB drugs are planning programmes to at least define the pharmacokinetic and safety profiles of their drugs in paediatric populations.

6.2 Goals of improved TB therapy

Improving TB therapy in both adults and children has four primary goals: shortening and simplifying treatment of active DS-TB to improve adherence and facilitate administration, while maintaining persistently high levels of efficacy and safety; improving therapy of both MDR- and XDR-TB by increasing the efficacy, safety and tolerability of drugs, as well as decreasing treatment duration, complexity and cost; improving treatment of TB patients co-infected with HIV by reducing DDIs between TB drugs and antiretroviral agents; and improving treatment of LTBI by shortening the duration of therapy and developing regimens equally efficacious against current DS and DR strains. However, achieving the goal of a short, simple regimen will require overcoming the phenomenon of persistence. A truly short treatment regimen for active TB could have a considerable impact on patients and public health systems, particularly in resource-limited, high-burden settings, by making adherence

to treatment substantially easier and by markedly reducing the selection pressure that causes the spread of new DR strains of *M. tuberculosis*.

Success will require the development of new, safe, cost-effective regimens of at least two to three new drugs that can be used concomitantly with anti-retroviral agents. The ultimate goal is an oral, once daily or even less frequent, treatment that works effectively against both DS- and DR-TB, needs to be taken for no more than two weeks, is affordable and can be made readily available.

6.3 Current status and future progress

For the first time, there is now a critical mass of new TB drugs in development: at least six distinct chemical classes, three of them with completely new mechanisms of action. Consequently, it is now feasible to begin the preclinical identification of optimized multidrug regimens that are equally effective against DS-, DR-, MDR- and XDR-TB and which may then be further developed in the clinic. Such regimens could initiate a true turnaround in TB treatment in the next 10–15 years.

6.3.1 Drug classes currently in clinical development

There are currently two broad classes of drugs in clinical development: those already used in first- or second-line TB treatment and those that have completely novel mechanisms of action. The former category includes the rifamycins, fluoroquinolones, and oxazolidinones; the latter category includes the nitroimidazoles, diarylquinolines and ethylene-diamines. These drugs have recently been reviewed in significant detail (*163–166*) and therefore will be described only briefly here.

6.3.2 Current first-and second-line TB drug classes undergoing new evaluation

Rifamycins: Rifamycins, the most effective of the currently available agents in killing slowly repli-cating or persistent *M. tuberculosis* (*167–169*), are being re-explored for use at relatively high doses. Recent data from mouse studies (*170*) suggest that high doses of rifamycins, especially rifapentine (a long half-life rifamycin), have the potential to reduce treatment duration to 3 months or less. The maximal tolerated doses of rifampicin and rifapen-tine have never been defined, so determining them is now a priority (*171–174*). Rifabutin exhibits fewer DDIs with antiretrovirals than rifampicin and rifap-entine and also deserves further evaluation.

Fluoroquinolones: The fluoroquinolones have marked efficacy against TB as well as relatively good safety and tolerability profiles (*175–183*). The most efficacious appear to be the 8-methoxyfluo-roquinolones, gatifloxacin and moxifloxacin, which are currently being evaluated in phase III trials for their ability to substitute for either ethambutol (gatifloxacin, moxifloxacin) or isoniazid (moxi-floxacin) in first-line TB treatment, and to shorten therapy from the current 6–9 months to 4 months (*184, 185*). Fluoroquinolones have also become an important component of MDR-TB treatment in some regions of the world. However, pre-existing levels of resistance to fluoroquinolones in some countries could, unfortunately, limit the utility of such a regimen in affected local populations (*186–188*).

Oxazolidinones: The oxazolidinones (a class of pro-tein synthesis inhibitors) have exhibited significant safety issues, particularly bone marrow suppres-sion and peripheral or optic neuropathy, associated with the use of their lead compound, linezolid(189). Linezolid has been used off-label, however, to treat both MDR- and XDR-TB(*190, 191*) and is currently being evaluated in a phase II trial for treatment of MDR-TB. Based on data from mouse studies (192), Pfizer has taken another oxazolidinone, PNU100480 (also known as sutezolid), into clinical development

for TB (*193*) and a phase II trial in pulmonary TB patients is under way. AstraZeneca has an oxazolidinone (AZD5847) in phase I development.

6.3.3 Drug classes with novel mechanisms of action

Nitroimidazoles: Two novel nitroimidazoles are currently in clinical development: OPC-67683 (delamanid), a nitro-dihydro-imidazooxazole, is being developed by Otsuka Pharmaceutical Company, and PA-824, a nitroimidazooxazine, by the Global Alliance for TB Drug Development (TB Alliance). Both are prodrugs and inhibit mycobacterial protein and lipid biosynthesis through mechanisms of action that have not yet been fully elucidated. Delamanid has completed a phase II trial in MDR-TB patients, where it was associated with an increase in sputum culture conversion at 2 months compared with placebo (each in addition to an optimized background regimen of current second-line drugs) (*194, 195*). Delamanid is currently being evaluated in a phase III trial in MDR-TB patients involving 6 months' treatment vs. placebo treatment in addition to an optimized second-line drug regimen. In late 2012, Otsuka submitted a conditional registration application for delamanid to EMEA.

PA-824 has been evaluated for safety, tolerability and pharmacokinetics in a number of phase I studies in healthy volunteers (*196, 197*) and extended early bactericidal activity (EBA) studies (*198*). The current phase of development is directed at evaluating its safety and efficacy in combination with other TB drugs in order to develop novel multidrug regimens that can shorten and simplify treatment of both DS- and DR-TB, including in HIV-co-infected patients. In a recently reported EBA study, Diacon et al. demonstrated that the combination of PA-824, moxifloxacin and pyrazinamide had greater activity over 14 days than bedaquiline (see section below on Diarylquinolines), bedaquiline plus pyrazinamide or bedaquiline plus PA-824, and comparable activity to that of the standard four-drug, first-line TB regimen. This combination therefore deserves further evaluation as a treatment for MDR-TB as

well as DS-TB, as it contains neither isoniazid nor rifampicin (*199*). The TB Alliance is currently evaluating this novel three-drug combination in a phase IIa, 2-month treatment trial in DS- and MDR-TB patients. Evaluation of safety and pharmacokinetics in paediatric populations will follow once adult safety and pharmacokinetic profiles are established.

Diarylquinolines: TMC207 (bedaquiline) was developed by Tibotec (now Janssen), a Johnson & Johnson subsidiary. In partnership with the TB Alliance, Janssen has established an integrated simultaneous TB drug development programme for DS- and MDR-TB and a joint discovery project to identify and develop "second generation" diarylquinolines. Bedaquiline acts by inhibiting the mycobacterial ATP-synthase proton pump (*200–202*). In a phase II trial, bedaquiline administered for 24 weeks with an optimized MDR-TB background regimen was well tolerated, resulted in faster culture conversion and had a higher sputum conversion rate (*203, 204*). A phase III trial is being planned to evaluate further its safety and efficacy in MDR-TB patients. In 2012, Janssen submitted applications to both the FDA and EMEA for accelerated or conditional approval, respectively, of bedaquiline. Guidelines for its compassionate use and expanded access are being developed by WHO.

Ethylenediamines: Sequella Inc. is developing the adamantylethanediamine, SQ109. SQ109's specific intracellular target has recently been reported to be MmpL3, a membrane transporter of trehalose monomycolate, through which it inhibits cell wall synthesis (*205, 206*). Sequella Inc. is currently conducting a 14-day, phase IIa, EBA study of SQ109 with and without rifampicin in DS-pulmonary-TB patients to further define the drug's safety, tolerability, and pharmacokinetic properties, in addition to its early bactericidal activity (*207, 208*).

6.4 The discovery and preclinical pipeline

Meeting the future treatment needs of TB patients and latently infected individuals requires a robust pipeline of projects and compounds in the early stages of drug development. The current pipeline ranges from whole-cell phenotypic screening efforts and target-based screens to projects focused on identifying and optimizing leads within specific, promising chemical classes, such as the diarylquinolines, nitroimidazoles, and rhiminophenazines. A small number of lead compounds are in formal preclinical development, including a benzothiazinone, a caprazene nucleoside, and new generation quinolones. A comprehensive overview of the current TB drug discovery and preclinical pipeline is available on the website of the Stop TB Working Group on New TB Drugs (www.newtbdrugs.org).

In recent years, funding for basic TB research has increased substantially (*209, 210*). Nevertheless, many fundamental aspects of TB pathogenesis have not yet been fully elucidated, and many more resources and efforts are needed to bring understanding to a level comparable to that for HIV (*210*).

Substantial challenges also continue to hamper the efforts of those working in TB drug R&D. These can be briefly summarized as follows:

- Inadequate understanding of the underlying molecular mechanisms of persistence and latency, and of the host–pathogen relationship.

- Lack of biomarkers of drug effect to facilitate efficient triage of candidates into late stage development and/or as surrogate markers of stable cure to shorten times of phase III trials.

- Long duration and high efficacy of current first-line treatment lead to lengthy and large, non-inferiority-design pivotal trials.

- Lack of clear regulatory guidance on the critical path to registration of novel, multidrug regimens containing more than one previously unregistered drug.

- Inadequate clinical and laboratory capacity to conduct registration-quality TB drug trials.
- The need for multidrug regimens, rather than single-drug treatments, substantially increases the hurdles to success by requiring discovery and development of several drugs without significant DDIs but with compatible pharmacokinetic properties.

A new initiative, the Critical Path to TB Drug Regimens (CPTR), was launched in 2010 by the Bill & Melinda Gates Foundation, the Critical Path Institute and the Global Alliance for TB Drug Development to further collaboration among pharmaceutical companies, government, regulatory agencies, multilateral agencies, civil society organizations, and other key stakeholders to accelerate the development of new, safe, effective and shorter TB treatment regimens (http://cptrinitiative.org/). The initiative is focusing on facilitating evaluation of different drug combinations instead of individual drugs. The barriers to scaling up this approach include funding, antitrust issues, and limited clinical trial capacities in HBCs.

The research priorities for new TB treatments are summarized in Table 6.1.

Table 6.1. **Drugs research priorities***

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Identify targets and pathways essential for persistence and/or latency, and determine genetic bases for drug resistance**	Basic and targeted research into *M. tuberculosis* biology and host–pathogen relationships.	Establishment of assays that will enable selection of novel compounds with treatment-shortening potential and improved understanding of drug-resistance mechanisms.	Current regimens for DS-TB, DR-TB and LTBI are too long, leading to poor adherence and drug-resistance.	Number of candidate targets validated through molecular genetics and/or animal models.
2	**Re-develop currently used TB drugs with strong animal model evidence that optimization could lead to substantial treatment-shortening (e.g. high-dose rifapentine)**	• Develop necessary preclinical toxicology and ADME data to support full clinical development. • Identify human maximally tolerated dose and optimize the dosing regimen. • Explore relevant DDIs in the clinic. • Perform phase I–III clinical development programme, as needed.	Optimization of current drugs for treatment-shortening potential: in particular, rifamycins, fluoroquinolones and oxazolidinones.	Several current drug classes have demonstrated previously untapped potential for treatment-shortening in one or more animal models.	• First-line treatment regimens of 4 months or less. • Shorter time to sputum conversion for MDR-TB and potentially XDR-TB (oxazolidinones).
3	**Identify and develop drugs with novel mechanisms of action and potential to meet the described target product profiles (oral, once daily or less frequent, safe, efficacious, treatment-shortening, few DDIs, low cost)**	For leads with appropriate preclinical data packages supporting an IND, conduct phase I–III clinical evaluation of new drugs to support global registration.	Shorter, safe, efficacious and affordable treatment for DS- and DR-TB, HIV-TB, and LTBI.	Drugs with novel mechanisms of action, in combination, would treat both DS- and M(X)DR-TB, and would be easily co-administered with ARVs with HIV-co-infected individuals.	• Number of new drugs registered. • Number of novel drug combinations registered.

*   For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
4	Identify and develop drugs with novel mechanisms of action that rapidly kill latent bacilli and/or prevent reactivation, and are easily delivered globally to large populations	• Conduct preclinical development to support an IND. • Conduct phase I to III development, including TB prevention trials in high-risk populations and LTBI trials with novel, short-duration regimens.	Short-duration regimens to treat latently infected individuals with goal of eradicating reservoir of future TB cases.	Approximately 2 billion people currently have LTBI, creating a huge reservoir of future active TB cases. Elimination of TB as a public health problem will require purging this reservoir effectively or preventing reactivation with drugs or vaccines.	• Registration of treatments for LTBI. • Ultimately, decreased incidence of active TB due to reactivation of LTBI.
5	Identify and qualify biomarker(s) of drug effect useful in: 1) early proof-of-concept trials to help sponsors quickly and effectively decide whether to take a candidate into late-stage development; and 2) shortening late-stage development timelines by substituting in pivotal trials for the clinical endpoint of stable cure vs. relapse	• Establish adequately large biobanks of clinically well-documented specimens collected from time of diagnosis through stable cure or relapse. • Use samples to discover and qualify potential biomarkers against: 1) the current standard of 2-month sputum conversion rate; and 2) gold standard' clinical endpoint of cure vs. relapse.	Biomarkers qualified (i.e. accepted by stringent regulatory authorities) useful in: 1) early clinical development to make rapid decisions as to whether to take a candidate or regimen into late-stage clinical development; and 2) pivotal trials to more quickly determine a drug's or regimen's efficacy and treatment-shortening ability.	Shortened clinical development timelines would enable drug candidates and regimens to be developed more cost-effectively and in a more rapid timeframe.	• Number of well-documented, high quality specimens collected and biobanks established. • Number of useful biomarkers qualified. • Extent to which early development pathways and phase III clinical trials are shortened.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
6	**Identify a critical path for development of novel TB treatment regimens containing more than one NCE, including innovative clinical trial designs and approaches to pharmacovigilance especially in high-burden settings, as appropriate**	Work with regulatory authorities, drug sponsors and experts in regulatory science, along with other key stakeholders, to develop the "critical path".	• Clear regulatory guidance issued by key regulatory agencies. Appropriate innovative trial designs published and successfully utilized in drug registration programmes. • Pharmacovigilance programmes for approved, novel regimens established.	Developing improved TB treatment regimens to meet the urgent medical needs will take several decades if each new drug must be developed and substituted into the current SOP one at a time. By establishing a critical path for development of novel regimens containing more than one NCE, this could be shortened to 6–10 years.	Time to registration of improved, novel, short-duration regimens that are safe and effective for treatment of DS- and DR-TB.
7	**Develop mouse models of TB and LTBI with human-like pathology (e.g. caseating granulomas in active TB) to facilitate evaluation of drug candidates and regimens**	• Explore various inbred mouse strains that display range of pathologies in response to M. tuberculosis infection. • Standardize models for use in drug development.	Standardized, mouse models of TB disease and LTBI with pathology similar to that displayed by humans — for use in preclinical efficacy evaluation of novel drugs and regimens for TB and LTBI.	• Current mouse models of TB display pathology different from that of humans and therefore may not accurately predict human efficacy. • Larger animals, such as guinea-pigs, rabbits and monkeys are not practical for widespread use due to expense and large drug requirements.	Standardized models shown to have human-like pathology of active TB and capable of establishing and reactivating LTBI that are predictive of drug efficacy in humans, including treatment duration for stable cure.
8	**Define attributable benefit of new treatment regimens, with respect to safety, tolerability and efficacy**	• Mathematical models. • Phase IV studies. • Assessments of patient perceptions of current and future TB drugs. • Better define private sector market and distribution channels.	Data on potential epidemiologic impact and cost-effectiveness of new regimens.	Would provide enhanced ability to compare new treatment regimens to current standards, and to each other, and to understand the markets, in order to influence adoption and help ensure availability of new regimens.	Number of studies conducted and used in influencing adoption decisions for new regimens and helping to ensure availability.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
9	**Assess global clinical trials capacity to conduct registration-quality TB drug trials and enhance capacity, as needed to fulfil needs of drug development programmes**	• Using a standardized tool, assess sites globally for ability to conduct ICH GCP-compliant phase II and III TB drug clinical trials (including EBA studies). • Enter data into broadly available, user-friendly databases. • Establish stable career paths for clinical research in high-burden settings. • Enhance capacity, build infrastructure, as needed.	• Up-to-date database of sites with detailed data on their capacity to conduct registration-standard phase II and III clinical trials. • Enhanced global capacity to support development of new TB treatments through conduct of registration-quality clinical trials.	Number of sites currently capable of conducting registration-quality TB drug trials is inadequate to meet the needs of the current and future TB drug pipeline.	• Number of sites globally available and capable of supporting TB drug registration programmes without significant delays for capacity-building or waiting for sites to become available. • Number of registration programmes conducted simultaneously and successfully without delays due to inadequate site capacity.
10	**Assess global laboratory capacity (especially mycobacteriology) to support registration-quality TB drug trials, and enhance capacity, as needed, to fulfil needs of drug development programmes**	• Using a standardized tool, assess laboratories globally for ability to conduct ICH GCP/GLP-compliant phase II and III TB drug clinical trials (including EBA studies). • Enter data into broadly available, user-friendly database. • Train microbiologists and establish stable career paths in high-burden settings. • Enhance capacity, build infrastructure, as needed.	• Up-to-date database of laboratories with detailed data on their capacity to support registration-standard phase II and III clinical trials. • Enhanced global capacity to support development of new TB treatments through conduct of registration-quality clinical trials.	Number of laboratories (biosafety level-3; ICH GLP) currently capable of supporting registration-quality TB drug trials is inadequate to meet the needs of the current and future TB drug pipeline.	• Number of laboratories (especially mycobacteriology) globally available and capable of supporting TB drug registration programmes without significant delays for capacity-building. • Number of registration programmes that are conducted simultaneously and successfully without delays due to inadequate lab capacity.

7 Intensified case-finding and TB infection control

7.1 Overview

The WHO Policy on Collaborative TB/HIV activities (2012) proposes collaborations at all levels between TB and HIV providers, reducing the burden of HIV in TB patients by HIV testing and provision of co-trimoxazole and antiretroviral therapy, and reducing the burden of TB in people living with HIV (PLHIV). The-last-mentioned activity has been termed the "three I's" strategy because it includes intensive case finding (ICF) for TB, provision of isoniazid prevention therapy (IPT) and infection control (IC).

7.2 Intensified case-finding

7.2.1 HIV

Under WHO's "three I's" strategy, individuals at risk for or living with HIV should regularly be screened for TB using a simple symptom screen, followed by diagnosis and treatment. This screen is not only an entry point for TB treatment but also for IPT and is an important component of IC (*211*). Despite this strategy and the relative simplicity of symptom screening, in 2009 only 5% of those estimated to need screening had actually been screened (*212*).

Several operational studies have been conducted in different institutional settings where individuals at risk of HIV are found. One of these settings is voluntary counselling and testing (VCT) centres. TB screening at the VCT centres in endemic countries has found a high rate of undiagnosed TB in all adults; with the rates in HIV-infected individuals being double those in individuals who were uninfected (*213–217*). The prevalence of TB is much higher among HIV-infected persons with a low CD4 count (*218*). Symptom screening in VCT centres could be used to rule out TB more easily than diagnosing it, and diagnosis using sputum culture rather than smear resulted in a high number of cases being found. One challenge for targeted ICF at the VCT centre is how to ensure a good referral network between the centre and a health facility able to follow up and diagnose TB and ensure that patients are

put on treatment. An operational research study from India showed that good referral networks can be implemented (*219*).

More research has been done on ICF in health-care facilities, including antenatal facilities offering prevention of mother-to-child transmission (PMTCT) programmes, as well as in HIV-care facilities. A study from a PMTCT clinic in Soweto, South Africa, among TST-positive women reported a prevalence of active TB of 11% (*334*). Another study in Soweto that used symptoms to screen pregnant women found a prevalence of culture-positive TB of 2.1% (*220*).

In HIV-care settings, outpatient facilities or inpatient wards, many patients are seen when they are already sick and in need of antiretrovirals. TB screening in these settings may yield a very high prevalence of undetected TB. For example, in a Rwandan inpatient facility, 7% of all inpatients and 22% of all HIV-positive patients were found to have TB using a combination of symptom screening and investigation according to national guidelines (*221*).

In an antiretroviral therapy (ART) clinic in South Africa, 25% of adults screened prior to receiving antiretrovirals were found to have culture-confirmed TB (*222*). Only 79% of those identified as having TB reported any of the symptoms usually used in screening. The combination of this symptom screen and a chest X-ray only had a sensitivity of 64% (specificity, 39%). Chest X-ray screening of PLHIV has been investigated in several HIV-care settings.

In the HIV setting, it is clear that whatever method of ICF is used, additional TB cases can be found if they are looked for. The screening algorithms that have been used are relatively simple but the follow-up and diagnosis are more challenging.

7.2.2 Prisoners and other high-risk groups

Many studies of case-finding have been conducted in prisons where the prevalence of TB is high. The strategies employed are similar to those used in HIV-care settings, where a symptom screen is the primary activity followed by more intensive investigation of individuals suspected to have TB based on their screening results. Case-finding identifies additional cases in prisons in better-resourced, lower TB prevalence settings (223) in higher TB prevalence settings (224, 225) and high HIV/TB co-infected settings (226–228).

Chest X-ray screening has also been conducted for many years among miners to detect occupational lung disease and TB. Several studies carried out in the gold mines of South Africa have demonstrated that this is effective for case-finding, but still misses a surprisingly large number of cases, especially where HIV prevalence is high (35, 229).

Other high-risk groups often screened for active TB are immigrants (230), household or other close contacts of active TB cases (231), health-care and laboratory staff (232, 233), and the homeless (234, 235). Guidelines for TB screening differ greatly among countries and programmes. A recent survey in Europe found that most countries screen contacts of smear-positive TB, but there is a wide discrepancy in the screening of all other high-risk groups (236).

Current tests for infection include the TST and IGRAs, which are discussed in more detail in chapter 5 on TB diagnostics. These tests do not distinguish between infection and disease but may be useful in low-prevalence settings for case-finding in nosocomial, school, prison or other outbreak settings.

7.2.3 Community-level intensive case-finding

Community-level case-finding is usually described as either active (ACF), where whole populations are screened, or enhanced (ECF), where increased community education and mobilization are employed and individuals are asked to present for further investigation. Much of the data about case-finding have come from subnational prevalence surveys that used an ACF methodology. A prevalence survey in Cape Town, South Africa, that examined the relative screening benefits of symptoms versus chest X-ray in a comparatively low HIV prevalence setting found that chest X-rays were more sensitive than symptom screening (237). Several recent studies of community-level case-finding have used the symptom screen followed by sputum smear (238–240), and all report that additional cases were found using this strategy. The wider availability of faster TB culture using liquid media has shown the additional benefit of using a more sensitive technology for case-finding at the community level, reporting low rates of smear positivity especially in high HIV prevalence settings (36, 241, 242). These studies have also prompted a reassessment of our current knowledge of the epidemiology of TB. In a study in Zambia, 20% of the cases identified using culture did not have symptoms of TB when questioned, some of these individuals developed symptoms when revisited some weeks later, and others who were minimally symptomatic appeared to have resolved when revisited (241). Several investigators have described similar cases of "subclinical" TB, but whether this is just a stage in the spectrum of infection and disease or a separate entity is not clear (25, 36, 243).

Prevalence surveys can also be used to understand the epidemiology of TB better. Several studies have found that the relative contribution of HIV to the prevalence of TB disease is less than its contribution to TB incidence, because TB rapidly progresses to a symptomatic stage that presents for treatment or results in death (36). Some individuals in the community may be responsible for transmitting large amounts of TB (244) and some of these individuals

will not be found by passive case-finding but would need ACF to identify and consequently eliminate them as a source of infection. The relative benefit of finding these cases will vary according to the epidemiology of TB and HIV in the community concerned.

One major research question is how to conduct community-level case-finding. For example, is ACF better than ECF, in terms of yield, cost and logistical feasibility? Two randomized trials have compared the two methods of case-finding. One of them, carried out in favelas in Brazil, found that door-to-door symptom screening identified more cases than a leaflet campaign combined with screening at the health centre (RR, 1.55; 95% CI, 1.10–1.99) (*245*). In Harare, a randomized trial of door-to-door case-finding compared with ECF using loudspeakers and a sputum collection van found the opposite: that ECF outperformed door-to-door case finding (adjusted RR, 1.5; 95% CI 1.1–2.0) (*36*). Overall, 41% of all smear-positive cases were detected by some means of case-finding, and the prevalence of TB in the communities where the trial took place was reduced by 40%. This study in Harare shows the potential benefit of community ECF, which may be more feasible than ACF. A recent study in Zambia and South Africa (the ZAMSTAR study) also evaluated the effectiveness of ECF using community education and sputum collection points, open access sputum examination at clinics and a schools' programme (*246*). It was found that the community-based ECF had no effect on either the prevalence of TB disease at the community level or on the incidence of its transmission in schoolchildren (*247*). In the same study, a strategy of using TB cases as an entry point to households at risk of TB and HIV with case-finding of household contacts of TB cases reduced both the prevalence of TB and the incidence of infection in schoolchildren, although the confidence intervals included 1. Understanding the different results from the studies on case-finding at community level is important and is currently under way assisted by mathematical modelling.

7.2.4 Methods of intensive case-finding

In most settings, a form of screening is used in ICF to identify individuals with the highest chance of having TB. A symptom screen has been the favoured method in recent times, although some studies have used the TST or chest X-ray in addition. Most screens are based on a constellation of cough, fever, night sweats and weight loss. The 2011 *WHO Guidelines for intensified case-finding* strongly recommend use of a standardized symptom screening algorithm for TB screening among HIV-infected persons (248). The algorithm (any cough, fever, night sweats or weight loss), based on a meta-analysis of 9626 patients enrolled in 12 studies conducted in different settings, has a sensitivity of 79.9%, specificity of 49% and negative predictive value of 97.7%. Questions on the feasibility and practical implementation of the algorithm remain, however. Chest X-ray screening in addition to symptom screening improved the sensitivity of symptom screening alone by 16% (*249*).

The use of a simplified screening tool, such as a symptom screen, also holds promise for wider application in overstretched health systems. Symptom screens can be successfully used by trained lay personnel, such as counsellors or TB treatment supporters, to identify those who need further investigation, thereby maximizing the number of individuals who can be screened by a given number of health-care workers.

Individuals identified by the screening process must be subjected to further investigation to diagnose TB (*250*). The greatest challenge in ICF is how to accurately diagnose TB, especially in HIV co-infected individuals, children and those with the extrapulmonary disease (see chapter 5). In essence, better diagnostic algorithms for specific circumstances are urgently needed, as are more rapid and sensitive diagnostic tests. Without these, ICF will not be successful.

7.3 Infection control

With the recognition of the increased risks posed by concurrent TB and HIV infection, and multiple nosocomial outbreaks of MDR- and XDR-TB, new emphasis has been placed on TB infection control (IC). The updated *WHO policy for TB infection control in health-care facilities*, congregate settings and households was published in 2009 (*251*). The policy consists of recommendations for national and subnational policies to provide direction and support activities at lower levels. For example, at the health facility level, it consists of four main activities: managerial activities, administrative controls, environmental controls and personal protection. Surveillance of health-care workers (HCWs) is of critical importance in the monitoring and evaluation of any infection control policy. Such surveillance has been extensively conducted in lower TB prevalence settings and is increasingly considered in lower income settings (*252, 253*). Most of this screening identifies infection rather than disease. The annual risk of TB infection attributable to the additional risk due to working as a health-care provider ranges from 0 to 11.3% (*252*). Many countries do not have a policy of routine surveillance of TB infection in HCWs, and most do not systematically record how many HCWs develop TB. HCWs living with HIV or any other disease that predisposes them to TB should be offered a package of care that includes ART and IPT (see chapter 8). Specific studies of the benefit of preventive therapy to HCWs have not been conducted and questions remain about the duration of treatment needed in this context.

At the health facility level, triage of infectious patients is essentially ICF as described above. Cough etiquette practices include covering the mouth and nose during coughing and sneezing using a tissue or surgical mask. However, there is little evidence for the effectiveness of these measures in reducing TB transmission, since they have mostly been implemented concurrently with other measures, usually as a combination of triage, separation and reduction of the time spent in overcrowded health facilities.

Environmental controls such as ventilation and upper-room UV light have been investigated more fully and various standards for number of air changes per hour and for disinfectant doses of UV light have been established (*254, 255*). However, many of these environmental control measures require investment and maintenance to be effective. Recent work from Peru has demonstrated that natural ventilation can be as effective as that of mechanical (*256*) and that upper-room UV lights and negative ionizers were effective in reducing the infection and disease rate in guinea-pigs subjected to air exhaust from TB wards (*257*).

Personal protection with respirators and high efficiency masks is appealing and theoretically makes sense, but there is little definitive evidence for their effectiveness (*258*).

7.3.1 Congregate settings

In congregate settings, the main recommendations are administrative controls. In such settings there is, however, little evidence for their effect on transmission rates.

7.3.2 Households

Recommendations for ICF at the household level have been proposed. However, because most of the transmission at household level probably occurs before a diagnosis of TB is made, IC may be relatively ineffective. The problem with recommendations at this level is that they potentially increase the stigma associated with TB in many parts of the world. Also, they are also often unfeasible unless TB programmes intend to take on the provision of better housing. In settings with a high prevalence of HIV infection, or where MDR- and XDR-TB are more common, formulating recommendations is even more challenging.

7.3.3 Effectiveness and cost-effectiveness

Four non-randomized studies in low- and middle-income countries have assessed the effectiveness of IC measures on HCWs. In Malawi, one such study (involving administrative control measures including cough etiquette and natural ventilation) found no effect on TB disease after 1 year, (*259*) but two others, in Thailand and Brazil, did show an improvement in infection rates (*260, 261*). Another study, from Brazil, concentrated on administrative measures plus some instruction on personal protection and also reported a reduction in infection rates among HCWs (*262*). A further study, an evaluation of the use of N95 respirator masks by HCWs in Brazil (*263*), found that the masks were infrequently used and that they did not fit adequately, thus potentially reducing protection. It was concluded that the masks were not cost-effective in low-resource settings and that administrative controls are more appropriate.

In higher-income countries, more studies of interventions have been conducted, consistently demonstrating significant reductions in transmission (*264*). In most studies, the interventions were implemented as a package, making it impossible to identify which individual measure had the greatest effect.

Table 7.1 summarizes the research priorities for ICF and IC

Table 7.1. Research priorities for intensified case finding and infection control *

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Improve understanding of the epidemiology of tuberculosis associated with HIV at community level**	• Prevalence surveys with culture (preferably liquid culture) as the endpoint. • Measures of transmission included. • Disaggregation by gender and socioeconomic status.	Accurate prediction of the burden of disease, relationship between transmission and disease in high HIV settings.	Some poorly funded surveys will not provide required level of evidence.	• Number of TB prevalence surveys assessing culture- proven disease in association with HIV. • Number of surveys/studies of TB transmission in association with HIV.
2	**Determine the most appropriate screening algorithm for case- finding in different settings**	• Systematic review. • Operational research using new screening algorithm.	• Screening algorithms recommended and adopted for use. • Screening algorithm evaluated in different settings.	Reluctance to initiate case- finding due to lack of know-how. Increasing numbers of studies with poor methodology.	• Systematic review published. • Number of screen-ing algorithms adopted. • Impact of use of algorithm on case detection and mortality
3	**Determine the efficacy and effectiveness of different case-finding strategies in different epidemiological settings**	Cluster randomized trials.	Effectiveness in terms of reduction in transmission and prevalence of disease.	Conflicting results of ZAMSTAR and DetecTB studies- need for other studies in epidemiological settings with different thresholds of TB/HIV prevalence.	• Number of trials reported from different settings. • Meta-analysis of trials
4	**Identify best case-finding strategy for different thresholds of TB incidence/ HIV prevalence, respectively, and related costs**	Mathematical modelling.	Recommendations for different epidemiological settings.	• Not all countries prepared to undertake mass case-finding. • some targeted interventions may be more appropriate.	• Recommenda-tions formulated and adopted for use in different epidemiological settings.
5	**Increased knowledge of annual risk of infection and incidence of TB in HCWs.**	Programmatic surveillance.	Information for policy-makers and advocacy for improved infection control.	Allows for monitoring of programme performance.	• Number of programmes reporting ARI or incidence of TB in HCWs.

*   For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
6	**Effect and costs of applying different parts of the infection control strategy on HCWs infection rates**	Operational research.	Better understanding of what works and what does not, and at what cost.	Ethically impossible to do trial but likely natural experiments as countries implement different strategies.	HCW infection rates decreasing.
7	**Comparison of natural and mechanical ventilation on TB transmission**	Cluster randomized trial.	Efficacy/ effectiveness and cost of different methods to reduce TB infection/ transmission in humans.	Important question for resource- limited settings on how to best use resources.	Number of trials reporting comparisons of natural vs. mechanical ventilation.
8	**Additional benefit of personal respiratory protection using different modalities including use of respiratory masks**	Randomized trials/ operational research.	Efficacy of different respiratory masks in different settings.	mportant question for best use of resources.	• Number of trials on efficacy. • Reduction in infection rates of HCWs.
9	**Effect of IC/ ICF strategies in non-health care facilities (congregate settings/ households)**	Longitudinal cohort studies with qualitative assessment of stigma.	Understanding of transmission dynamics, optimal frequency of screening, benefits, risks and costs of different strategies.	If most transmission is occurring before diagnosis, current IC recommendations are not useful, but may be improved by addition of periodic case-finding.	• Number of studies contributing to understanding. • Reduction of infection rates in close contacts of patients with ac- tive tuberculosis.
10	**Effect and optimum duration of IPT in HCWs**	Randomized trials.	Clear policy recommendations for HCWs with and without HIV in high TB prevalent settings.	No data available in high TB prevalence settings with a high proportion of HCWs living with HIV.	Evidence- based policy recommendation on the use of IPT in HCWs in different HIV prevalent settings.

8 Management and prevention of HIV-associated TB

8.1 Overview

Infection with HIV increases the risk of progressing to TB disease following infection with *M. tuberculosis* (*265*) and the risk of recurrence, primarily due to re-infection, following treatment completion (*39, 40, 42*). HIV impairs the host's immune response, predisposing to smear-negative, extrapulmonary and disseminated forms of TB (*266–268*). This contributes to diagnostic delays, increased morbidity and higher case-fatality rates from TB among the HIV-infected (*87*). TB may induce progression of HIV disease with declines in CD4 counts (*269*). The DOTS strategy alone is insufficient to control TB in high HIV-prevalence settings and additional strategies are therefore essential to meet the global targets for TB control. Such strategies are broadly classified into those for preventing TB among people living with HIV (PLHIV) and those for reducing morbidity and mortality in those with HIV-associated TB. Some interventions for preventing TB infection (ICF and IC) are discussed in chapter 7.

The present chapter reviews progress and the current state of understanding about the optimum case management for patients with HIV-associated TB and the use of antiretroviral therapy (ART) and isoniazid preventive therapy (IPT) as interventions for prevention of TB in PLHIV.

8.2 Management of HIV-associated TB

The following key interventions are required for the optimum case management of patients with HIV-associated TB:

- Rapid ascertainment of HIV-associated TB cases through provider-initiated HIV testing and counselling of TB patients as well as routine TB screening among PLHIV.
- Use of short-course anti-TB treatment.
- ART.
- Administration of co-trimoxazole preventive therapy (CPT).
- Detection and management of MDR-TB.

8.2.1 Case ascertainment

Because of low rates of HIV testing among TB patients and of TB screening among PLHIV, a huge burden of HIV-associated TB remains unascertained (*212*). This is compounded by the poor performance of TB diagnostics in HIV-infected patients. In addition, a great burden of TB disease exists among patients who have yet to access the health-care system.

HIV testing of TB patients is needed to activate an adjunctive package of clinical treatment and care as well as to prevent onward transmission of HIV. Where HIV prevalence among TB patients exceeds 5%, provider-initiated testing and counselling for HIV (PITC) must be offered as a routine service, whereby patients undergo HIV testing as part of the diagnostic work-up unless they specifically "opt-out". Although this has been shown to be feasible and acceptable in various settings (*270*), less than half of TB patients underwent HIV testing in 2007; however, this proportion shows that considerable progress has been made since 2002 (*212*). Operational research is needed to define how this testing can be best streamlined at an early stage into TB diagnosis and registration efforts, and how it can be facilitated by shifting the task of HIV testing to trained lay counsellors in settings where there are human resource shortages.

8.2.2 Anti-tuberculosis treatment

For cases of drug-sensitive TB (DS-TB) involving HIV-infected patients, treatment regimens that contain a rifamycin for the entire treatment period have better outcomes than those that only include rifampicin for the initial 2 months (*271*). Use of a continuation phase of once-weekly rifapentine and isoniazid for HIV-infected patients and of twice-weekly rifampicin-containing regimens in those with advanced immunodeficiency have each been associated with an increased risk of relapse and drug resistance (*272, 273*). The 2010 revision of WHO's *Treatment of tuberculosis guidelines* recommend daily TB treatment, both in the intensive

phase and in the continuation phase; where daily dosing is not feasible, at least three times weekly dosing is recommended (*274*). Furthermore, it is recommended that the duration of TB treatment for patients with HIV-associated TB should be the same as that for HIV-negative TB patients (*274*). Recurrence rates may be substantially reduced through use of concurrent ART (*39*) or of secondary IPT (*275*). The optimum management strategy needed to minimize recurrence rates using these combined interventions remains to be defined. In the longer term, new drugs and regimens are much needed (see chapter 6).

8.2.3   Antiretroviral therapy

TB case fatality rates in Africa are 16–35% among HIV-infected patients not receiving ART and 4–9% among non-HIV-infected individuals (*276*). ART is associated with significant reductions in mortality risk (64–95% in adjusted analyses) (*277*).

In 2010, WHO issued new guidelines recommending that ART should be initiated as soon as possible, and within 8 weeks of starting TB treatment (*278*). Subsequent randomized controlled trials have shown that those individuals with the lowest CD4 cell counts (<50 cells/μL) should be prioritized for ART within the first 2 weeks to reduce mortality risk, and that those with higher counts can delay for up to 8 weeks (*279–281*). The concurrent use of TB treatment and ART is complicated by pharmacokinetic interactions, with induction of the CYP450 enzymes by rifampicin leading to increased metabolism of non-nucleoside reverse transcriptase inhibitors (NNRTIs) and protease inhibitors (PIs) (*271, 282*). Although the findings of pharmacokinetic studies vary, rifampicin appears to cause a more significant reduction in plasma levels of nevirapine than of efavirenz (*282, 283*). The largest observational study to date comparing use of nevirapine and efavirenz suggested that the latter may have superior virological efficacy and lower toxicity (*284*). Efavirenz-based ART is currently the preferred first-line regimen for patients receiving rifampicin-based TB treatment, except for pregnant women in the first

trimester. Alternatives include either nevirapine-based or triple-nucleoside regimens. However, data from randomized controlled trials are lacking, including trials that involve special groups such as pregnant women, children and patients co-infected with hepatitis B or C.

For patients receiving PI-based second-line ART and who need TB treatment, current recommendations are to use additional ritonavir-boosting of lopinavir or saquinavir with close monitoring (*270, 271, 282*). However, rifabutin causes far less hepatic enzyme induction than rifampicin and has emerged as an important alternative in HIV-infected patients with a potentially important role among those receiving PI-based second-line ART. However, PIs are also potent inhibitors of the hepatic enzyme CYP3A, requiring reduction of the dose of rifabutin and its administration on alternate days (*270, 271, 282*). Research is needed to determine whether rifabutin can be incorporated into fixed-dose combinations (FDCs) and whether it can be readily used in public health programmes. Studies are also needed to assess the efficacy and interactions of newer classes of ART drugs, such as entry inhibitors and integrase inhibitors, when used in combination with TB treatment.

The optimal time to start ART during TB treatment remains an important management issue. Early mortality rates are extremely high among patients waiting to start ART in resource-limited settings (*50*), and a study in South Africa found that the mortality risk among patients initiating ART after completing 6 months' TB treatment was double that of patients starting within the first 3 months of TB treatment (*285*). Early initiation of ART in patients with HIV-associated TB increases the risk of immune reconstitution inflammatory syndrome (IRIS) but reduces mortality (*268, 269*).

8.2.4 Management of HIV-associated MDR-TB

Mortality rates in patients with HIV-associated MDR-TB are very high, even in specialized treatment programmes (*54*). Management of such patients requires the use of a minimum of four effective drugs (*286*), and there are few data to inform the optimum use of ART in patients with HIV-associated MDR-TB (*287*).

Second-line TB drugs are associated with considerable adverse effects and some of their toxicity profiles overlap with those of antiretroviral drugs (*288*). Also, the pharmacokinetic interactions between these agents and antiretroviral drugs are incompletely characterized.

In view of the very high mortality risk associated with co-infections of this type, ART should probably be initiated early in the course of MDR-TB treatment. A study from South Africa showed that among HIV-infected patients with XDR-TB early mortality on treatment was significantly improved with ART use (*289*). The extremely high pill burden, high risk of TB IRIS and prolonged duration involved in concurrent therapy require very high doctor and patient commitment and adherence support (*290*). Operational research is therefore needed to define optimum approaches.

8.3 Prevention of HIV-associated TB

8.3.1 Antiretroviral therapy

ART is associated with marked reductions (54–92%) in TB incidence (*277*), which occurs among patients with all WHO stages of TB disease and with a wide range of baseline CD4 cell counts (*277, 291*). Reductions in TB incidence are time-dependent, with the most benefit occurring during the first 2–3 years of starting ART (*292, 293*). TB rates are directly related to the change in absolute CD4 cell counts during ART (*294*). Despite the major risk reduction, TB rates during long-term ART remain several-fold higher

than those observed in non-HIV-infected people in the same communities (*292-296*). Adjunctive TB prevention interventions are therefore needed during long-term ART such as IPT and serial ICF, and studies are needed to define these interventions.

Empirical observations and mathematical modelling suggest that ART, as currently used for patients with advanced immunodeficiency, is likely to have only limited impact on population-level TB incidence in settings with high HIV prevalence (*297*) since much of the HIV-associated TB has already occurred before patients start ART (*293, 298*). A move towards much earlier initiation of ART, such as within the "test and treat" strategy (universal HIV testing and immediate initiation of ART), could have substantially more impact (*299*), and evaluation of such an intervention is needed in sentinel study communities. However, it remains likely that multiple synergistic strategies will be most effective.

8.3.2 Isoniazid preventive therapy

IPT has proven preventive efficacy in both non-HIV-infected and HIV-infected individuals (*300, 301*). Systematic review of randomized trials found that the combined efficacy of all TB preventive regimens in HIV-infected adults was 36% (95% CI, 19–49%), with the greatest reduction being among those with positive TST reactions. A single randomized placebo-controlled trial involving HIV-infected children in South Africa found that isoniazid halved mortality and reduced TB risk by approximately 70% (*302*).

The duration of protection derived from IPT is much shorter (1.0–2.5 years) for HIV-infected patients in high TB burden settings (*303, 304*), possibly due to exogenous re-infection (*305*), and progression of immunodeficiency may increase susceptibility to exogenous re-infection or reactivation of incompletely eradicated LTBI. There is evidence that IPT decreases overall mortality among adults with a positive TST response (*301*).

In a study from Botswana, the TB preventive effect of a 6-month course of IPT among HIV-infected

patients was lost within 6 months of stopping treatment, whereas TST-positive patients receiving IPT for 36 months exhibited marked suppression of TB rates (*306*). In a South African trial, an intention-to-treat analysis found no additional preventive benefit but a higher cumulative toxicity among those receiving continuous treatment (*307*). Similarly, a study from India failed to demonstrate a benefit from prolonged therapy (*308*). No studies have yet determined the relative merits of intermittent repeated 6-month courses of IPT versus continuous treatment. The WHO IPT guidelines for PLHIV make a conditional recommendation for at least 36 months of IPT (*248*).

Although a positive TST is very strongly predictive of which patients will derive benefit from IPT, the requirement for assessment of TST status was recognized as a major obstacle to the implementation of IPT because the practicalities and logistics of performing a TST mean that it is not feasible to carry out this test in many settings. The WHO IPT guidelines for PLHIV therefore do not require a TST before starting IPT. Research is needed to make assessment of TST status operationally more feasible or to find a suitable replacement assay. An additional major operational barrier is the need to exclude active TB prior to commencing IPT (see chapter 5).

 Studies have shown the beneficial impact of secondary IPT following completion of TB treatment (*275, 309, 310*). Since many patients receiving ART have already had TB, these findings add to the rationale for use of isoniazid among those receiving ART. No data are yet available on the risk and appropriate preventive therapy for HIV-infected individuals exposed to patients with MDR-TB, and studies are needed in this regard. New prophylactic drugs are needed against both DS- and DR-TB infection.

The population-level impact of widespread use of IPT in countries with high HIV prevalence is unclear. Modelling studies suggest the impact may be limited in the long-term (*311–313*), but large-scale, community-based efficacy studies are needed to verify this, although such studies can only be undertaken

in the context of ART scale-up. Poor uptake of IPT requires operational research to identify barriers and develop appropriate models of delivery. Additional research needs to explore alternative preventive treatment regimens and strategies, including the use of new anti-TB drugs as they emerge.

8.3.3 Complementary roles of IPT and ART

ART reduces TB risk by immune recovery, whereas IPT reduces the burden of viable mycobacteria. These mechanisms are potentially complementary and may form a rational basis for the combined use of these interventions (*314*).

Data from Brazil, South Africa and Botswana indicate an additive effect of IPT when administered prior to or during ART (*306, 315, 316*). In view of the low negative predictive value of TB screening just prior to beginning ART among those with low CD4 cell counts, it has been proposed that IPT might preferably be initiated in such patients only after they complete the first few months of ART. Randomized controlled trials and operational research are needed to explore these issues and clearly define optimum and feasible prevention strategies for use of these interventions in adults and in children.

Research priorities for HIV associated TB are listed in Table 8.1.

Table 8.1. Research priorities for HIV associated TB *

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	To determine optimal first-line ART regimens for use during concurrent rifampicin-containing TB treatment	Randomized controlled trials.	Determination of comparative safety and efficacy of different regimens.	The evidence base for prioritizing one regimen over the other is weak.	• Clear prioritization of different regimens based on safety and efficacy. Number of countries recommending use of high priority regimens.
2	To determine appropriate weight- and age-adjusted doses of ART drugs in children receiving rifampicin	Pharmacokinetic studies in children.	Development of recommendations for paediatric ART drug dosing during rifampicin-containing TB treatment.	Data to inform correct dosing of ART drugs during TB treatment in children are very scarce.	• Clear guidelines for age- and weight-adjusted dosing of ART in children. • Number of country programmes using these guidelines.
3	Development of optimum formulations and FDCs of ART and TB drugs to facilitate adherence to concurrent treatment in adults and children	Pharmacokinetic studies.	Identification of ART FDCs with satisfactory pharmacokinetic profiles during concurrent rifampicin therapy.	Use of FDCs can greatly simplify concurrent TB treatment and ART for patients and health care workers, improving adherence and reducing the risk of development of drug resistance.	• Number of ART and TB treatment FDCs developed. • Number of country programmes using these FDCs.
4	Identification of optimal dosing and frequency of rifabutin-containing TB treatment during protease-inhibitor-based ART, permitting development of rifabutin-based FDC TB treatment	Pharmacokinetic studies in adults and children.	Identification of appropriate rifabutin dosing schedules and development of FDCs for use during PI-based ART.	Rifabutin-based TB treatment is preferred during PI-based ART but optimal dosing schedules and development of FDCs are needed to permit operationalization.	• Development of guidelines on use of rifabutin- and rifabutin-containing FDCs. • Number of country programmes including use of rifabutin.

* For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
5	**What is the optimal TB preventive therapy in terms of efficacy, safety, tolerability and duration of protection for use in HIV-infected adults, children, pregnant women and those with underlying hepatic disease?**	Randomized control trials involving each of these key patient groups.	Determination of comparative safety and efficacy of different regimens in key patient groups.	Although TB preventive therapy is an intervention of proven efficacy, uptake remains poor. Optimization of recommendations would enhance uptake.	• International and national recommendations for use of TB preventive therapy. • Number of patients receiving preventive therapy.
6	**To determine whether TB preventive therapy provides additional TB risk reduction in adults and children receiving ART and to determine the safety and tolerability of concurrent therapy**	Randomized control trial of isoniazid vs. placebo in adults and children receiving ART.	Determination of whether the benefits of IPT (any reduction in TB rates) outweigh potential additional toxicity and reduced adherence and increased cost.	TB rates during long-term ART remain >5-fold higher than background, and adjunctive interventions are needed.	• Development of international and national policies for use of TB preventive therapy during ART.
7	**To determine the optimal screening algorithm to be used across settings with different TB burden to permit safe initiation of TB preventive therapy**	Meta-analysis of TB screening studies from diverse countries and settings.	Identification of optimum screening strategy based on efficacy and utility in diverse settings.	Perceived inability to reliably exclude active TB in HIV-infected persons is a major stumbling block to scale up of TB preventive therapy.	• Development of international and national recommendations for screening. • Number of patients screened for TB using the optimized algorithm.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
8	**To determine the optimum operational models to scale up implementation of TB preventive therapy**	Operational research within different settings and health-care structures.	Identification of feasible approaches to IPT delivery and monitoring.	Despite policy recommendations of an effective intervention, uptake remains extremely poor.	• Identification of optimum models of scale-up for use in different settings. • Rate of scale-up of TB preventive therapy.
9	**Identification of the impact of second-line TB drugs on the pharmacokinetics of ART drugs**	Pharmacokinetic studies.	Characterization of pharmacokinetic interactions between second-line TB drugs and NNRTI-based and PI-based ART.	Very little is known about the impact of second-line drugs used in the treatment of MDR-TB on ART drug levels.	• International and national recommendations for use of second-line TB drugs during ART. • Numbers of patients receiving appropriate second-line TB therapy during ART.
10	**To define whether early ART initiation (e.g. using the 'test and treat' strategy) reduces individual TB risk and improves community TB control in high HIV prevalence communities**	Observational study in sentinel cohorts and communities.	Assessment of the feasibility and cost-effectiveness of early ART and the "test and treat" strategy for TB control.	Cumulative TB risk is strongly dependent upon cumulative time spent at low CD4 counts.	• Impact of high coverage early ART on TB notification rates.

9 Operational research

9.1 Overview

Finding solutions to some of the technical challenges that currently impede the diagnosis, treatment and prevention of TB could have a significant impact on improving case detection and cure rates. Strengthening general health systems, particularly in areas such as human resources, laboratory infrastructure, infection control, drug forecasting, data monitoring, supervision and quality assurance, would enable better care to be delivered at all levels, and in so doing improve TB control efforts. The need for stronger health systems is reflected in the WHO Stop TB Strategy (*317*), the Stop TB Partnership's Global Plan to Stop TB 2011 – 2015 (*6*) and in the *Stop TB Policy Paper: contributing to health system strengthening* (*318*). The key issue is how to make the conceptual framework and policies contained in the policy documents work in practice; research to this end would be highly beneficial. This chapter summarizes the progress, challenges and research results in implementing DOTS, TB/HIV integration, managing MDR-/XDR-TB as well as paediatric TB from an operational perspective and identifies priorities for operational research.

9.2 Improving the screening and diagnosis of TB, including drug resistance

9.2.1 Operational aspects of intensified screening for TB

Much of the morbidity and mortality from TB in people living with HIV (PLHIV) could be prevented by application of the WHO's "three I's" (ICF, IC and IPT, see chapters 5 and 8).

9.2.2 Increasing case detection of smear-positive primary TB

Current case detection strategies for smear-positive primary TB are challenged by the difficulties faced by patients in accessing health facilities, weak laboratory infrastructure and poor recording practices. The following new approaches could resolve some of these problems and lead to increased case detection rates. First, front-loaded microscopy, involving collection and examination of two sputum specimens on the first day a patient presents, should be used in combination with immediate referral and treatment of those found to be smear-positive (*103*). Second, fluorescence microscopy of sputum smears with inexpensive battery-powered LEDs (*97, 319, 320*), should be introduced to improve diagnostic sensitivity and efficiency;. Third, TB programmes should ensure that all patients diagnosed with smear-positive primary TB in public or private laboratories are entered into TB treatment registers, so that they are included in case-finding and treatment outcome cohort reports – currently 5–15% of patients with smear-positive primary TB are not entered into such registers and fail to be counted (*321, 322*). Operational research focusing on these three approaches would provide a sound evidence base for scale-up.

9.2.3 Point-of-care test for TB

Diagnosis of smear-negative TB in both adults and children continues to be problematic (*323*). A new point-of-care (POC) diagnostic test for use in health centres as well as in district hospitals could revolutionize the diagnosis and management of TB. However, the efficacy and feasibility of any new such test will first need to be gauged in the field before being incorporated into clinical practice in a better planned and regulated way than is the case with current POC tests. There is also a need to provide national policy-makers with better decision-making models.

9.2.4 More rapid and easier diagnosis of MDR-TB

Patients at risk of MDR-TB (all patients who fail treatment, those on retreatment after defaulting, and contacts of index MDR-TB patients) must have their sputum examined as early as possible for culture and drug-sensitivity testing. This should increase case detection of MDR-TB; however, for it to work, central reference laboratories need to be equipped with reliable, simple-to-use technologies, be staffed by skilled technicians, and investments be made in a composite package that includes sputum specimen transportation and timely feedback of results to peripheral clinics. New technologies, such as Cepheid's Xpert® MTB/RIF (reviewed in chapter 5), need to be piloted in district hospitals (*324*), because only when drug resistance testing is decentralized will this diagnostic tool become more widely available for the patients in need. Operational research is required to determine the diagnostic and treatment outcomes in TB suspects who are managed by the routine health service using a newly introduced screening diagnostic algorithm compared to the established algorithm which had been used previously.

9.2.5 Improved TB infection control in health-care settings

Exposure to tubercle bacilli in health-care facilities almost certainly accounts for an appreciable proportion of TB infection risk for patients, especially PLHIV who repeatedly attend clinics for chronic care. WHO's policy on TB infection control (251) has recently been updated, and observational research needs to be carried out to assess how far its recommendations have been adopted and implemented. More research is also required to evaluate the role of environmental control measures in reducing or preventing nosocomial TB transmission in crowded health-care settings. Furthermore, research is needed to better define the risk posed by integrated TB and HIV services at the individual clinic level and, importantly, to find ways to better implement joint HIV–TB care. Infection control is discussed in more detail in chapter 7.

9.3 Better prevention of TB, especially in people living with HIV

9.3.1 Isoniazid preventive therapy

A number of important operational research questions are linked to the implementation of IPT. Better and easier ways of determining the presence or absence of LTBI would improve the targeting of this intervention in PLHIV, and more sensitive and specific ways of diagnosing active TB in PLHIV urgently need to be identified.

Providing IPT requires capacity to screen patients for TB and adequate infection control, otherwise there may be a paradoxical increase in the risk of acquiring TB. The "three I's" are thus highly interdependent and complementary, a fact that requires that they be integrated into pre-ART and ART care at both facility and community level. How this is best done needs to be answered through careful operational research.

Data from Brazil (*315*) and South Africa (*316*) suggest that use of ART and IPT together may synergistically result in a highly significant decline in risk of active TB. The patient on ART is in structured care, and proper administration of IPT could be managed, even within the context of a busy clinic. Combined use of IPT and ART should be studied both through observational cohort studies, randomized controlled trials and operational research. If IPT is implemented to scale, any development of isoniazid resistance should be carefully monitored, since this will occur if patients with unrecognized active TB slip through the net. Isoniazid mono-resistance might compromise the effectiveness of the first-line anti-TB regimens, and surveillance of this is important. Use of IPT in PLHIV has been discussed in more detail in chapter 8.

9.3.2 Universal annual HIV testing and immediate/early start of ART

Although use of ART results in major reductions in TB rates in treatment cohorts, the impact on TB control at the community level is limited because so many PLHIV, particularly in sub-Saharan Africa, start treatment with CD4 counts below the level at which TB usually presents (*50, 325*).

Mathematical modelling of frequent testing of all adults for HIV and immediate introduction of ART if they are diagnosed as HIV positive (*299*) suggests that such an approach could have a major impact on reducing TB incidence in PLHIV. A major research priority is to determine how to use ART to achieve maximum prevention of HIV infection as well as of HIV-associated TB. The mathematical models need to be tested for efficacy, feasibility, safety, impact and cost (*326*). Four important operational issues require attention. First, community acceptability and protection of human rights need to be high on the agenda, as the proposed approach is aimed at relatively healthy individuals who might not obtain any immediate direct benefit from early ART (public health benefit versus the individual patient benefit). Second, the ART regimen has to be safe and tolerable for the many patients who are asympto-

matic and have high CD4 counts, so that adherence is maximal and the potential risk of drug resistance minimal. Third, with human resources so lacking in Africa, attention must be paid to delivery systems and particularly to identifying who will administer the therapy. Finally, simple, standardized monitoring and reporting systems will be vital for surveillance of outcomes and ensuring reliable ART drug forecasting and supplies.

9.4 Improved treatment for previously treated TB and MDR-TB

9.4.1 More rational retreatment for failures and recurrent TB

Retreatment for TB has for long been a neglected area in the control of the disease (*327*). Issues related to the treatment of patients previously treated for TB remain under-examined and under-resourced, despite the estimated one million people every year receiving or who are in need of a retreatment regimen (*328*). Although national and international guidelines stipulate that all previously treated TB patients should submit sputum for culture and drug-sensitivity testing before starting retreatment, this rarely happens. The 2010 WHO Treatment of Tuberculosis Guidelines recommend that in settings where drug susceptibility testing is not available, patients who fail category-1 treatment (new cases with either sputum-positive pulmonary TB, smear-negative pulmonary TB with extensive parenchymal involvement, severe extrapulmonary TB or severe concomitant HIV disease) should be started on empirical treatment for MDR-TB, while those who return after relapse or default can be put on a retreatment regimen (*329*).. The rollout of Cepheid's Xpert® MTB/RIF test may go a long way to improving detection of rifampicin resistance among both new and retreatment cases so that more timely, effective and safer retreatment options can be given to patients who develop recurrent TB.

9.4.2 Standardized short-course treatment for MDR-TB

Given the urgent need to increase access to treatment for MDR-TB, careful evaluation of treatment strategies is vital to ensure that the most effective and feasible approaches are implemented, particularly in the low-income settings where most cases of MDR-TB are found. Although several new drugs with novel mechanisms of action for the treatment of MDR-TB (see chapter 6) will soon become available for public use, there is still a need to assess the tolerability, safety and efficacy of shorter regimens (such as the Bangladesh 9-month regimen (*330*) under close-to-routine programme conditions. A combination of proper clinical and cohort observational studies is needed to address this issue.

Table 9.1 lists the research priorities for operational research.

Table 9.1. TB operational research priorities *

No.	Research priority	Research methods	Outcome	Justification
1	**Most feasible and optimal ways to undertake intensified TB case-finding in communities and in PLHIV**	Operational research.	Better screening for TB in the community and among PLHIV as part of the "three I's".	Large proportion of unrecognized TB in the community, and minimal implementation of TB screening in PLHIV.
2	**New approaches for increasing case detection of smear-positive PTB through front-loaded microscopy and LED fluorescence microscopy**	Operational research.	Increased case detection.	Case detection rates still not reaching global target of 70%.
3	**POC test for TB**	Development of a test based on molecular methods, which then needs to be tested for efficacy and feasibility.	New diagnostic tool for TB.	Poor case detection and inaccurate diagnosis of smear-negative TB in adults and children.
4	**More rapid and easier diagnosis of MDR-TB**	Efficacy and feasibility studies by clinical and operational research.	New diagnostic tool for MDR-TB.	Poor case detection of MDR-TB.
5	**Tuberculosis IC**	Models of TB infection control that can be implemented (operational research).	Better TB infection control in high-risk settings.	Large amount of TB nosocomial transmission in health facilities.
6	**Implementation of IPT in pre-ART and ART settings**	Operational research.	Increased scale-up of IPT.	Minimal implementation of IPT globally.

* For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Outcome	Justification
7	**Universal annual HIV testing and early start of ART to reduce HIV/TB**	Cluster randomized controlled trials, and observational cohort studies.	Elimination of HIV/TB in high burden areas.	HIV/TB burden still high despite good collaborative efforts.
8	**More rational retreatment regimen for patients who fail or develop recurrent TB after first-line treatment**	Randomized controlled trials or observational cohort studies.	More rational and safe retreatment regimen than is currently provided.	Concerns that the current WHO retreatment regimen may amplify drug resistance in those previously treated with rifampicin/isoniazid regimens.
9	**Simple, standardized treatment regimen for MDR-TB**	Randomized controlled trials or observational cohort studies.	Standardized regimen that is acceptable to patients and can be used in resource-poor settings.	Better treatment outcomes for MDR-TB.
10	**Evidence to show synergies between disease-specific programmes and general health systems**	Observational and descriptive research.	Better understanding of the relationship between disease-specific programmes and health systems.	Brings data to the current debate.

10 Gender and social determinants of TB

10.1 Social determinants of TB

In order to accelerate progress towards reaching the MDGs and Stop TB targets for TB, sociobehavioural and gender issues should be addressed and prioritized for research. Social science research plays two important roles in TB control: identifying social determinants of the disease or treatment outcomes; and identifying social and behavioural interventions to reduce its burden. The social determinants of TB also influence patients' health seeking behaviour and providers' behaviour. A framework for integrating TB social science into TB biomedical research is shown in Figure 10.1.

10. 2 TB and gender

TB is one of the leading causes of death among women of reproductive age in low-income countries (*331*). Following the MDGs' emphasis of the importance of gender equality and the fact that they specifically set targets for reducing death among women and children, the *Global tuberculosis control report 2011* included estimates of TB incidence in women but stressed the need for improved reporting disaggregated by sex (*332*). The Global tuberculosis control report 2012 reports a global male: female ratio of 1.9 for smear-positive pulmonary TB and for the first time includes mortality rates separately for women (*4*). However, disaggregated reporting varied significantly, particularly in HBCs, with data on smear-negative TB often not available. This indicates that many national TB control programmes are still not gender sensitive in their reporting and that gender issues have not been mainstreamed into the planning and implementation of TB programmes.

Table 10.1 lists the research priorities for gender and social determinants of TB.

Fig. 10.1. Framework for social science research in tuberculosis

Social science, health system, epidemiological, health economic and clinical research to identify risk factors of TB and the interventions to reduce TB mortality and TB morbidity at the individual and community level

General population

Social interventions to reduce risk of exposure and infection

Structural and biomedical interventions to reduce risk of exposure and infection

Social determinants

Biological factors

Diagnosis tools for LTBI

Social interventions for promoting access to diagnosis for latent TB infection

Latent TB infection

Social interventions for promoting access to diagnosis for latent TB infection

Vaccine, drugs, other innovations to reduce biological risk of TB disease

Social determinants

Biological factors

Tools for diagnosis TB

Social interventions for promoting access to diagnosis

Active TB disease

Social interventions for promoting access and adherence to treatment of TB, TB/HIV co-infection and MDR-TB

Drugs and other innovations to cure TB

Social determinants

Biological factors

Outcomes: cured, died, treatment failure and relapse

Basic science research and research for development of innovations and biotechnology interventions to reduce TB mortality and TB morbidity at the individual and community level
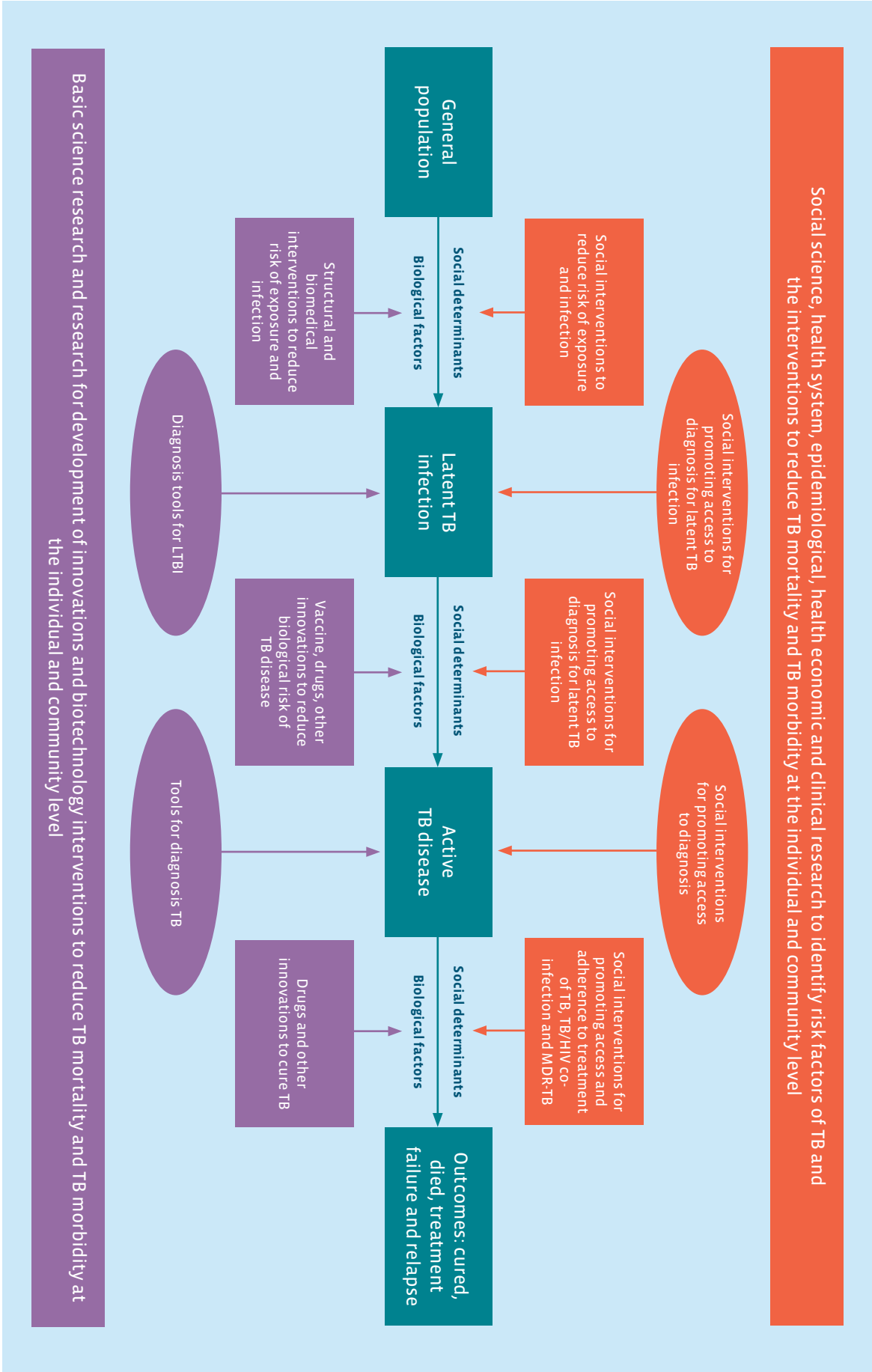
Table 10.1. Research priorities for gender and social determinants of TB *

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
1	**Identifying barriers to implement IPT in PLHIV**	Randomized controlled Survey of knowledge, attitude and willingness to implement IPT among HIV clinic staff. In-depth interviews with national and provincial managers of HIV and TB programmes. Focus group discussions with district TB/HIV programme coordinators.	• Reasons for slow progress in implementing IPT will be identified. • Practical recommendations to enhance IPT implementation will be obtained.	Although WHO and UNAIDS issued the policy statement on IPT for PLHIV in 1998 over a 10-year period no progress was made to implement IPT. In 2008 WHO recommended scaling up the three I's for PLHIV, including IPT. However, implementing IPT within countries' programme conditions is still not in place and the reasons are not known.	• Level of knowledge and attitude about the benefit of IPT for PLHIV. • Acceptability by policy-makers and service providers to implement IPT.
2	**Research to enhance contact tracing and provision of IPT to children under 5 years living with household members with active pulmonary TB**	• Focus group discussion with various stakeholder groups: paediatricians; district TB coordinators; TB clinic staff; community health volunteers; TB patients; and mothers with young children. • Use of focus group discussion results to plan and to implement CT and IPT.	Practical recommendations for implementation of CT and IPT.	• Children under 5 years who are close contacts with household members with pulmonary TB at high risk of TB infection and disease. • CT and IPT services not widely implemented.	• Awareness and acceptability of CT and IPT among stakeholders. • Increased coverage of CT and IPT.
3	**To study beliefs and practice about TB infection control in the household and in the community setting and to identify infection control interventions to minimize the risk of transmission**	Ethnographic study to gain insight into people's perspectives about infection control. Observational study of TB households.	To develop culturally sensitive guidelines for implementing TB infection control in the household and community settings.	TB transmission occurs in the household before diagnosis and treatment for TB is started. Most studies on TB infection control are conducted in health-care settings. Little is known about TB infection control practices in household and community settings.	• Proportion of TB households who open their house windows. • Proportion of TB patients who practice cough etiquette in the household and community.

* For an explanation of the abbreviations and acronyms, see p. 8-9

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
4	**Research to enhance TB case-finding and TB prevention through MCH services (ante-natal care, postpartum care, family planning and baby vaccination services) including PMCT**	Focus group discussion with stakeholders: obstetricians, paediatricians and staff in-charge of MCH and PMCT; district TB coordinators; community health volunteers; and women from MCH and PMCT	Barriers to implementing TB case-finding and IPT from the stakeholders' perspective identified and incorporated into planning and designing MCH, PMCT and TB linked-programmes.	Mortality of infants whose mothers have TB is significantly higher than those of mothers without TB. Undiagnosed mothers can transmit TB to other mothers and infants utilizing the same health facility. MCH and PMCT service present opportunities for detection and prevention of TB. Information about health workers and mother perception and acceptance of TB screening and IPT are not known (e.g. pregnant mother or mothers with breast feeding may not accept TB drugs or IPT).	• Number of women in the MCH services that receive TB screening. • Number of HIV positive mothers without active TB receiving IPT. • Number of infants whose mothers have active TB and are receiving IPT.
5	**Evaluate strategies to reduce TB stigma, especially among women**	• Focus group discussion with young men; young or single women mothers-in-law; religious leaders; and health workers. • Designing and implementing the intervention to reduce stigma. • Evaluation of the intervention.	Development of interventions to reduce TB stigma for women.	• Since 1995, research on TB stigma, especially from India and Muslim countries with high TB burden, has consistently reported serious negative social and health impacts of TB stigma in women. Single women with TB cannot get married. Married women are forced to divorce if they are diagnosed to have TB. TB stigma delays women seeking care and causes non-adherence to TB treatment. • Interventions which specifically address stigma in women are lacking.	• Number of married female patients who are forced to divorce after being diagnosed with TB • Level of stigma-tization against women with TB before and after stigma-reducing interventions. • Proportion of women who delay accessing TB service. • Proportion of women who default from treat-ment.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
6	**Research to identify barriers to treatment adherence and interventions to improve adherence to TB treatment, particularly among patients co-infected with TB and HIV**	• Focus group discussions with patients with good and poor adherence; TB clinic staff; and HIV clinic staff. • Patient medical record analysis. • Develop and test strategies to improve adherence. • Evaluate the impact of the intervention on adherence.	Barriers to treatment adherence will be identified. Interventions to promote adherence will be developed.	Adherence to both TB and HIV treatment are socially and biologically complex. Many studies investigated either adherence to TB treatment alone or adherence to ARV. Little is known about adherence to both TB and HIV treatment in patients with TB and HIV co- infection.	• Adherence rate. • Default rate.
7	**Research to identify and test the interventions to improve HCWs attitude and willingness to care for TB patients, including marginalized population and patients with MDR-TB or XDR-TB**	• Extensive literature reviews on HCW's attitude and motivations to work in resource-limited-countries and attitude towards TB and TB/HIV patients. • Focus group discussion with HCWs working in TB clinic. • TB patients; and policy-makers.	Interventions to improve HCWs' attitude and performance in line with patient-centred approach will be identified and tested.	The international standard of TB care promotes a patient-centred approach which requires mutual respect between patients and care providers. However, research to improve staff's attitude and TB care according to patient-centred approach is lacking.	• Staff's attitude to-wards TB patients before and after the interventions. • Staff's awareness about human right before and after the interven-tions. • Patients' satisfac-tion with staff performance.
8	**Improving TB case detection in health-care facilities and community settings by providing culturally sensitive instructions for sputum submission**	• Focus group discussions with women, men and HCWs to understand the community's perception of sputum collection. • Develop an instruction manual for sputum collection and evaluate in the community, especially among women. Evaluate the impact of the instruction manual by analysing the laboratory register.	The research results can be used for planning and developing culturally- and gender-sensitive instruction manuals for sputum collection for use in health facility and community settings.	• Poor quality of sputum samples undermines TB diagnosis. Proper instruction for sputum collection increased TB case detection, especially among women. To date, studies have been carried out in clinic settings. • In order to promote TB detection through community- based care, the social and cultural meaning of "sputum" should be studied from the gender perspective.	• Proportion of good sputum sub-mission from men and women • Ratio of smear positivity between men and women.

No.	Research priority	Research methods	Expected outcome	Justification	Selected indicators
9	**Research to understand what motivates community and patient volunteers to take part in TB care**	• Analysis of volunteers' profiles. • Focus group discussion with the volunteers who contribute to TB care for more than 3 years. • In-depth interviews with volunteers who leave and those who continue their volunteer work.	Process of recruiting community and patient volunteers, including motivations and volunteers' best practices will be identified. The results may be applicable to other settings.	Empowering patients and the community is one of the six components of WHO's Stop TB Strategy. Community and patient volunteers play a significant role in community-based TB care. However, little is known about what motivates them. In resource-limited settings, it is important to identify and implement appropriate alternative incentives that can motivate community and patient volunteers to participate in TB care in a sustainable manner.	• Attrition rate of volunteers. • Volunteers' satisfaction. • Number of TB patients supported by volunteers (for case detection, for counselling, treatment support).
10	**Research to enhance access to TB diagnosis and to complete TB treatment for marginalized and vulnerable populations (homeless and street children, indigenous minorities, illegal immigrants, prisoners, drug users, sex workers)**	• Ethnographic study to understand perspectives about TB. • Identify interventions to promote case-finding and ensure treatment adherence. • Evaluate the intervention.	Interventions to enhance access to diagnosis and treatment of these populations will be obtained and may guide policy.	In both high and low TB- endemic countries TB incidence is significantly higher in marginalized populations or among those with low social and economic status. Most TB studies report problems encountered by these populations but little is known about what interventions could improve access to diagnosis and lead to completion of treatment.	• Duration and reasons for patient delay. • Proportion of the marginalized population who complete treatment.

11 Consolidation of priority areas of research

11.1 Stakeholder consultation

A list of the top 10 priority research areas was sent electronically to international funders (3 per stakeholder), researchers (1 per stakeholder), NGOs (2 per stakeholder), civil society (1 per stakeholder), UN agencies (8 per stakeholder) and national control programmes in HBCs (2 per stakeholder) for review and comment. Responses were received from 5/17 (29.4%) of the stakeholders.

The research priorities for TB were further validated through a national stakeholder consultation held on 15 September 2010 in Manila, Philippines. A total of 55 representatives from a wide range of stakeholders participated in the consultation, including government TB programmes, NGOs, a TB/MDR-TB patient advocate, the Philippine Coalition against Tuberculosis, the HIV/AIDS council, epidemiologists and clinicians, including infectious disease specialists, pulmonologists and dermatologists, social scientists and representatives from the other major Philippine island clusters that were predominantly front-line health-care providers. Participants were divided into four small groups with Disease Reference Group (DRG) members embedded in each group as a resource. The four groups reported back on their deliberations to all stakeholders. The local stakeholders placed a high premium on operational research and strengthening of health systems, while a low premium was placed on biomarkers and vaccines. From the local stakeholders' perspective, the key messages were as follows: effective new tools (diagnostics, drugs and vaccines) should be affordable and accessible; a trans-disciplinary approach to neglected tropical diseases is required; and health systems strengthening should be viewed as mutually related and beneficial vis-à-vis disease control efforts.

11.2 Top 10 research priorities

The final consolidated list of top 10 research priorities for tuberculosis, which were selected as described in chapter 2 and then updated following input from local, regional and international stakeholders, is shown in Table 11.1.

11.3 Gaps and limitations

This report has a number of gaps and limitations. The overview of thematic areas and the process of identifying gaps in knowledge and research priorities were based on expert perspective and opinion rather than systematic reviews. Also, the focus was more on downstream (development of new tools and implementation) than upstream research (basic science) required for discovery and development of new drugs, diagnostics, and vaccines. Furthermore, it does not adequately address paediatric, MDR- or extrapulmonary TB, but rather is complementary to the WHO/Stop TB Partnership research priorities report An international roadmap for tuberculosis research: towards a world free of tuberculosis (*333*), which does address many of these gaps

11.4 Lessons learnt

Convening an expert committee, writing the thematic reviews, identifying priorities and soliciting stakeholder input is a long process. Validation of research priorities by key stakeholders is an important but challenging procedure. The process of harmonizing the research priorities identified by the DRG with those of other groups setting research priorities requires careful consideration and coordination.

Table 11.1. Consolidated list of the 10 top research priorities for tuberculosis *

Priority	Expected outcomes	Notes
Improve diagnostics for infection, disease and drug resistance for TB, especially POC tests.	Development of new technologies that will lead to improved individual and public health outcomes and equity that will be required to meet MDG targets.	Top priority is rapid POC tests, including rapid diagnosis of drug resistance and extrapulmonary TB. Focus on tests that also work in children and HIV-infected persons. Algorithms need consideration.
Develop improved treatment and prevention regimens (based on current and new drugs) for TB.	Development of new technologies that will lead to improved individual and public health outcomes and equity that will be required to meet MDG targets.	Development of a more rational TB retreatment regimen is a priority: short, tolerable, effective regimens for MDR-TB are required.
Identify and validate biomarkers that facilitate development of vaccines, diagnostics and drugs for TB.	Development of new technologies that will lead to improved individual and public health outcomes and equity.	—
Develop novel vaccines and optimize current vaccines for TB.	Development of new technologies that will lead to improved individual and public health outcomes and equity that will be required to meet MDG targets.	This will be helped by developing a blueprint for development.
Evaluate and optimize strategies to improve case-finding and reduce barriers to treatment access for TB.	Improved individual and public health outcomes and equity that will be required to meet MDG targets.	Should include optimization of screening algorithms, evaluation among vulnerable populations and cost-effectiveness analyses. Evaluations to improve case finding should include existing and new tools.
Increase understanding of the burden of disease, the modes of transmission and the impact of public health interventions for TB.	Guide public health interventions to facilitate meeting MDG targets.	—
Increase understanding of the pathogenesis of TB to fuel discovery of drugs, vaccines and diagnostics	Improve probability of success for development of new technologies that will lead to improved individual and public health outcomes and equity.	—
Optimize implementation of preventive therapy for TB, including drug-resistant TB.	Improved individual and public health outcomes and equity that will be required to meet MDG targets.	There should be a focus on community-based management of MDR-TB.
Evaluate strategies to strengthen health systems to support control of TB.	Improve cost– benefit and address issues of equity, gender and social justice	Should include financing, human resources, service delivery and management.
Evaluate and optimize new and current strategies to quantify, prevent and minimise disability and stigma resulting from TB.	Address issues of equity, gender and social justice.	—

*   For an explanation of the abbreviations and acronyms, see p. 8-9

12 Conclusions

TB is an infectious disease of poverty that accounts for a large proportion of the global burden of disease and mortality. Transformational research on TB is required in order to drive discovery and to develop new tools. Once effective new tools are developed, it will be important to evaluate methods for effective implementation and determine their impact on TB control. By identifying priorities validated by key stakeholders, we hope that funders, researchers and policy-makers will adopt them and that this will lead to increased funding and transformational research. It is envisioned that the results will transform policies and practice that will accelerate progress towards achieving the MDGs and the objectives of the Stop TB Partnership for eliminating TB as a global health threat by 2050.

References

1.   *Ten year vision and strategy*. Geneva, Special Programme for Research & Training in Tropical Diseases, 2007 (TDR/GEN/06.5/EN/Rev.2).

2.   *Priority research questions for TB/HIV in HIV-prevalent and resource-limited settings.* Geneva, World Health Organization, 2010 (WHO/HTM/TB/2010.8).

3.   *An international roadmap for tuberculosis research: towards a world free of tuberculosis.* Geneva, World Health Organization, 2011.

4.   *Global tuberculosis report 2012.* Geneva, World Health Organization, 2012.

5.   *Multidrug and extensively drug-resistant TB (MD/XDR-TB): 2010 global report on surveillance and response*. Geneva, World Health Organization, 2010.

6.   *The Global Plan to Stop TB 2011–2015. Transforming the fight towards the elimination of tuberculosis.* Geneva, World Health Organization, 2010 (WHO/HTM/STBI 2012.2).

7.   Bernatas JJ et al. *Comparison du risque annuel d'infection tuberculeuse (1994–2001) auprès d'enfants scolarisés à Djibouti: limites méthodologiques et intérêt épidémiologique dans un contexte hyperendémique.* [Comparison of annual risk for tuberculosis infection (1994–2001) in school children in Djibouti: methodological limitations and epidemiological value in a hyperendemic context]. *Médecine Tropicale (Revue du Corps de santé colonial),* (Mars) 2008,68(6):611–616.

8.   Winje BA et al. School-based screening for tuberculosis infection in Norway: comparison of positive tuberculin skin test with interferon-gamma release assay. *BMC Infectious Diseases*, 2008, 8(140):887–893.

9.   Pulickal AS, Fernandez GV. Comparison of the prevalence of tuberculosis infection in BCG vaccinated versus nonvaccinated school age children. *Indian Pediatrics*, 2007, 44(5):344–347.

10.   Friedman LN et al. Tuberculosis screening in alcoholics and drug addicts. *American Review of Respiratory Disease*, 1987, 136(5):1188–1192.

11.   Paul EA et al. Nemesis revisited: tuberculosis infection in a New York City men's shelter. *American Journal of Public Health*, 1993, 83(12):1743–1745.

12.   Zolopa AR et al. HIV and tuberculosis infection in San Francisco's homeless adults. Prevalence and risk factors in a representative sample. *Journal of the American Medical Association*, 1994, 272(6):455–461.

13.   Nyamathi AM et al. A randomized controlled trial of two treatment programs for homeless adults with latent tuberculosis infection. *International Journal of Tuberculosis and Lung Disease*, 2006, 10(7):775–782.

14.   Mahomed H et al. Are adolescents ready for tuberculosis vaccine trials? *Vaccine*, 2008, 26(36):4725–4730.

15.   Wood R et al. Changing prevalence of tuberculosis infection with increasing age in high-burden townships in South Africa. *International Journal of Tuberculosis and Lung Disease*, 2010, 14(4):406–412.

16.   Reichler MR et al. Treatment of latent tuberculosis infection in contacts of new tuberculosis cases in the United States. *Southern Medical Journal*, 2002, 95(4):414–420.

17.   Alseda M, Godoy P. Factors associated with latent tuberculosis infection in the contacts of tuberculosis patients. *Gaceta Sanitaria/SESPAS*, 2004, 18(2):101–107.

18.   Arend SM et al. Double-blind randomized phase I study comparing rdESAT-6 to tuberculin as skin test reagent in the diagnosis of tuberculosis infection. *Tuberculosis (Edinburgh, Scotland)*, 2008, 88(3):249–261.

19. Abu-Raddad LJ et al. Epidemiological benefits of more-effective tuberculosis vaccines, drugs, and diagnostics. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106(33):13980–13985.

20. Gagneux S. Fitness cost of drug resistance in *Mycobacterium tuberculosis*. *Clinical Microbiology and Infection*, 2009; 15(Suppl 1):66–68.

21. Luciani F et al. The epidemiological fitness cost of drug resistance in Mycobacterium tuberculosis. *Proceedings of the National Academy of Sciences of the United States of America*, 2009, 106(34):14711–14715.

22. Khue PM et al. A 10-year prospective surveillance of *Mycobacterium tuberculosis* drug resistance in France 1995–2004. *European Respiratory Journal*, 2007, 30(5):937–944.

23. Wright A et al. Epidemiology of antituberculosis drug resistance 2002-07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. *Lancet*, 2009, 273(9678):1861–1873.

24. Casal M et al. A case–control study for multidrug-resistant tuberculosis: risk factors in four European countries. *Microbial Drug Resistance*, 2005, 11(1):62–67.

25. Cohn DL. Subclinical tuberculosis in HIV-infected patients: another challenge for the diagnosis of tuberculosis in high-burden countries? *Clinical Infectious Diseases*, 2005, 40(10):1508–1510.

26. Moore DA et al. Microscopic-observation drug-susceptibility assay for the diagnosis of TB. *New England Journal of Medicine*, 2006, 355(15):1539–1550.

27. Moniruzzaman A et al. Impact of country of origin on drug-resistant tuberculosis among foreign-born persons in British Columbia. *International Journal of Tuberculosis and Lung Disease,* 2006, 10(8):844–850.

28. Pablos-Mendez A et al. Nonadherence in tuberculosis treatment: predictors and consequences in New York City. *American Journal of Medicine*, 1997, 102(2):164–170.

29. Espinal MA et al. Determinants of drug-resistant tuberculosis: analysis of 11 countries. *International Journal of Tuberculosis and Lung Disease*, 2001, 5(10):887–893.

30. Sharma SK et al. Clinical and genetic risk factors for the development of multi-drug resistant tuberculosis in non-HIV infected patients at a tertiary care center in India: a case–control study. *Infections, Genetics and Evolution*, 2003, 3(3):183–188.

31. Lee JH, Chang JH. Drug-resistant tuberculosis in a tertiary referral teaching hospital of Korea. *Korean Journal of Internal Medicine*, 2001, 16(3):173–179.

32. Becerra MC et al. Tuberculosis burden in households of patients with multidrug-resistant and extensively drug-resistant tuberculosis: a retrospective cohort study. *Lancet*, 2011, 377(9760):147–152.

33. Lazarus JV et al. Tuberculosis–HIV co-infection: policy and epidemiology in 25 countries in the WHO European region. *HIV Medicine*, 2008, 9(6):406–414.

34. Ahmed AB et al. The growing impact of HIV infection on the epidemiology of tuberculosis in England and Wales: 1999-2003. *Thorax*, 2007, 62(8):672–676.

35. Corbett EL et al. Human immunodeficiency virus and the prevalence of undiagnosed tuberculosis in African gold miners. *American Journal of Respiratory and Critical Care Medicine*, 2004, 170(6):673–679.

36. Corbett EL et al. Epidemiology of tuberculosis in a high HIV prevalence population provided with enhanced diagnosis of symptomatic disease. *PLoS Medicine*, 2007, 4(1):e22.

37.   Corbett EL et al. Comparison of two active case-finding strategies for community-based diagnosis of symptomatic smear-positive tuberculosis and control of infectious tuberculosis in Harare, Zimbabwe (DETECTB): a cluster-randomised trial. *Lancet*, 2010, 376(9748):1244–1253.

38.   Panjabi R, Comstock GW, Golub JE. Recurrent tuberculosis and its risk factors: adequately treated patients are still at high risk. *International Journal of Tuberculosis and Lung Disease*, 2007, 11(8):828–837.

39.   Golub JE et al. Recurrent tuberculosis in HIV-infected patients in Rio de Janeiro, Brazil. *AIDS*, 2008, 22(18):2527–2533.

40.   Charalambous S et al. Contribution of reinfection to recurrent tuberculosis in South African gold miners. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(8):942–948.

41.   Nahid P et al. Treatment outcomes of patients with HIV and tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2007, 175(11):1199–1206.

42.   Sonnenberg P et al. HIV-1 and recurrence, relapse, and reinfection of tuberculosis after cure: a cohort study in South African mineworkers. *Lancet*, 2001, 358(9294):1687–1693.

43.   Nunn AJ et al. Role of co-trimoxazole prophylaxis in reducing mortality in HIV infected adults being treated for tuberculosis: randomised clinical trial. *BMJ*, 2008, 337:a257. doi: 10.1136/bmj.a257

44.   Ciglenecki I et al. Population differences in death rates in HIV-positive patients with tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 2007, 11(10):1121–1128.

45.   Haar CH et al. Tuberculosis drug resistance and HIV infection, the Netherlands. *Emerging Infectious Diseases*, 2007, 13(5):776–778.

46.   Cain KP et al. The epidemiology of HIV-associated tuberculosis in rural Cambodia. *International Journal of Tuberculosis and Lung Disease*, 2007, 11(9):1008–1013.

47.   Zwang J et al. Trends in mortality from pulmonary tuberculosis and HIV/AIDS co-infection in rural South Africa (Agincourt). *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2007, 101(9):893–896.

48.   Duarte EC et al. Factors associated with deaths among pulmonary tuberculosis patients: a case–control study with secondary data. *Journal of Epidemiology and Community Health*, 2009, 63(3):233–238.

49.   Gandhi NR et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of tuberculosis. *Lancet*, 2010, 375(9728):1830–1843.

50.   Lawn SD et al. Early mortality among adults accessing antiretroviral treatment programmes in sub-Saharan Africa. *AIDS*, 2008, 22(15):1897–1908.

51.   Gandhi NR et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet*, 2006, 368(9547):1575–1580.

52.   Singh JA, Upshur R, Padayatchi N. XDR-TB in South Africa: no time for denial or complacency. *PLoS Medicine*, 2007, 4(1):e50.

53.   Andrews JR et al. Multidrug-resistant and extensively drug-resistant tuberculosis: implications for the HIV epidemic and antiretroviral therapy rollout in South Africa. *Journal of Infectious Diseases*, 2007, 196(Suppl 3):S482–490.

54.   Wells CD et al. HIV infection and multidrug-resistant tuberculosis: the perfect storm. *Journal of Infectious Diseases*, 2007, 196(Suppl 1):S86–107.

55. van Rie A et al. Childhood tuberculosis in an urban population in South Africa: burden and risk factor. *Archives of Diseases in Childhood,* 1999, 80(5):433–437.

56. Murray CJ, Styblo K, Rouillon A. Tuberculosis in developing countries: burden, intervention and cost. *Bulletin of the International Union against Tuberculosis and Lung Disease*, 1990, 65(1):6–24.

57. Salazar GE et al. Pulmonary tuberculosis in children in a developing country. *Pediatrics*, 2001, 108(2):448–453.

58. Middelkoop K et al. Rates of tuberculosis transmission to children and adolescents in a community with a high prevalence of HIV infection among adults. *Clinical Infectious Diseases*, 2008, 47(3):349–355.

59. Berggren Palme I et al. Detection of *Mycobacterium tuberculosis* in gastric aspirate and sputum collected from Ethiopian HIV-positive and HIV-negative children in a mixed in- and outpatient setting. *Acta Paediatrica*, 2004, 93(3):311–315.

60. Shingadia D, Novelli V. Diagnosis and treatment of tuberculosis in children. *Lancet Infectious Diseases*, 2003, 3(10):624–632.

61. Walls T, Shingadia D. Global epidemiology of paediatric tuberculosis. *Journal of Infection*, 2004, 48(1):13–22.

62. Odhiambo JA et al. Tuberculosis and the HIV epidemic: increasing annual risk of tuberculous infection in Kenya, 1986-1996. *American Journal of Public Health*, 1999, 89(7):1078–1082.

63. Range N et al. Trend in HIV prevalence among tuberculosis patients in Tanzania, 1991–1998. *International Journal of Tuberculosis and Lung Disease*, 2001, 5(5):405–412.

64. Phypers M. Pediatric tuberculosis in Canada. *Canada Communicable Disease Report*, 2003, 29(16):139–142.

65. Colditz GA et al. The efficacy of bacillus Calmette–Guérin vaccination of newborns and infants in the prevention of tuberculosis: meta-analyses of the published literature. *Pediatrics*, 1995, 96(1 Pt 1):29–35.

66. Colditz GA et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *Journal of the American Medical Association*, 1994, 2(9):698–702.

67. Brewer TF. Preventing tuberculosis with bacillus Calmette–Guérin vaccine: a meta-analysis of the literature. *Clinical Infectious Diseases*, 2000, 31 (Suppl 3):S64–67.

68. Revised BCG vaccination guidelines for infants at risk for HIV infection. *Weekly Epidemiological Record*, 2007, 82(21):193–196.

69. Barreto ML, Pereira SM, Ferreira AA. BCG vaccine: efficacy and indications for vaccination and revaccination. *Journal de Pediatria (Rio de Janeiro)*, 2006, 82(3 Suppl):S45–54.

70. Crampin AC, Glynn JR, Fine PE. What has Karonga taught us? Tuberculosis studied over three decades. *International Journal of Tuberculosis and Lung Disease*, 2009,13(2):153–164.

71. Barreto ML et al. Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: Second report of the BCG-REVAC cluster-randomised trial. *Vaccine*, 2011, 29(31):4875–4877.

72. Sterne JA, Rodrigues LC, Guedes IN. Does the efficacy of BCG decline with time since vaccination? *International Journal of Tuberculosis and Lung Disease*, 1998, 2(3):200–207.

73. Kagina BM et al. Delaying BCG vaccination from birth to 10 weeks of age may result in an enhanced memory CD4 T cell response. *Vaccine*, 2009, 27(40):5488–5495.

74.   Hesseling AC et al. BCG and HIV reconsidered: moving the research agenda forward. *Vaccine*, 2007, 25(36):6565–6568.

75.   Mansoor N et al. HIV-1 infection in infants severely impairs the immune response induced by Bacille Calmette–Guérin vaccine. *Journal of Infectious Diseases*, 2009, 199(7):982–990.

76.   Lin MY, Ottenhoff TH. Host–pathogen interactions in latent *Mycobacterium tuberculosis* infection: identification of new targets for tuberculosis intervention. *Endocrine, Metabolic and Immune Disorders – Drug Targets*, 2008, 8(1):15–29.

77.   Govender L et al. Higher human CD4 T cell response to novel *Mycobacterium tuberculosis* latency associated antigens Rv2660 and Rv2659 in latent infection compared with tuberculosis disease. *Vaccine*, 2010 Dec 10, 29(1):51–57.

78.   Locht C et al. Heparin-binding hemagglutinin, from an extrapulmonary dissemination factor to a powerful diagnostic and protective antigen against tuberculosis. *Tuberculosis (Edinburgh, Scotland)*, 2006, 86(3–4):303–309.

79.   Gilleron M et al. Diacylated sulfoglycolipids are novel mycobacterial antigens stimulating CD1-restricted T cells during infection with *Mycobacterium tuberculosis*. *The Journal of Experimental Medicine*, 2004, 199(5):649–659.

80.   Layre E et al. Mycolic acids constitute a scaffold for mycobacterial lipid antigens stimulating CD1-restricted T cells. *The Journal of Biological Chemistry*, 2009, 16(1):82–92.

81.   Kamath AT et al. Protective anti-mycobacterial T cell responses through exquisite in vivo activation of vaccine-targeted dendritic cells. *European Journal of Immunology*, 2008, 38(5):1247–1256.

82.   Hawkridge T et al. Safety and immunogenicity of a new tuberculosis vaccine, MVA85A, in healthy adults in South Africa. *Journal of Infectious Diseases*, 2008, 198(4):544–552.

83.   Scriba TJ et al. Distinct, specific IL-17- and IL-22-producing CD4+ T cell subsets contribute to the human anti-mycobacterial immune response. *Journal of Immunology*, 2008, 180(3):1962–1970.

84.   Hanekom WA et al. Immunological outcomes of new tuberculosis vaccine trials: WHO panel recommendations. *PLoS Medicine*, 2008, 5(7):e145.

85.   Beveridge NE et al. Immunisation with BCG and recombinant MVA85A induces long-lasting, polyfunctional *Mycobacterium tuberculosis*-specific CD4+ memory T lymphocyte populations. *European Journal of Immunology*, 2007, 37(11):3089–3100.

86.   Rook G.A, Lowrie DB, Hernandez-Pando R. Immunotherapeutics for tuberculosis inexperimental animals: is there a common pathway activated by effective protocols? *Journal of Infectious Diseases*, 2007,196(2):191–198.

87.   Perkins MD, Cunningham J. Facing the crisis: improving the diagnosis of tuberculosis in the HIV era. *Journal of Infectious Diseases,* 2007,196(Suppl 1):S15–27.

88.   Pai M, O'Brien R. New diagnostics for latent and active tuberculosis: state of the art and future prospects. *Seminars in Respiratory and Critical Care Medicine*, 2008, 29(5):560–568.

89.   Sreeramareddy CT et al. Time delays in diagnosis of pulmonary tuberculosis: a systematic review of literature. *BMC Infectious Diseases*, 2009, 9:91.

90.   Perkins MD, Roscigno G, Zumla A. Progress towards improved tuberculosis diagnostics for developing countries. *Lancet*, 2006, 367(9514):942–943.

91.   Perkins MD, Small PM. Partnering for better microbial diagnostics. *Nature Biotechnology*, 2006, 24(8):919–921.

92. Minion J, Zwerling A, Pai M. Diagnostics for tuberculosis: what new knowledge did we gain through The International Journal of Tuberculosis and Lung Disease in 2008? *International Journal of Tuberculosis and Lung Disease*, 2009, 13(6):691–697.

93. Fontela PS et al. Quality and reporting of diagnostic accuracy studies in TB, HIV and malaria: evaluation using QUADAS and STARD standards. *PLoS One*, 2009, 4(11):e7753.

94. Pai M, Ramsay A, O'Brien R. Evidence-based tuberculosis diagnosis. *PLoS Medicine*, 2008, 5(7):e156.

95. Schunemann HJ et al. GRADE: assessing the quality of evidence for diagnostic recommendations. *American College of Physicians Journal Club*, 2008, 149(6):2.

96. *New laboratory diagnostic tools for tuberculosis control*. Geneva, World Health Organization and Stop TB Partnership, 2008.

97. Steingart KR et al. Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review. *Lancet Infectious Diseases*, 2006, 6(9):570–581.

98. Steingart KR et al. Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review. *Lancet Infectious Diseases*, 2006, 6(10):664–674.

99. Steingart KR, Ramsay A, Pai M. Optimizing sputum smear microscopy for the diagnosis of pulmonary tuberculosis. *Expert Review of Anti-Infective Therapy*, 2007, 5(3):327–331.

100. Mase SR et al. Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review. *International Journal of Tuberculosis and Lung Disease*, 2007, 11(5):485–495.

101. Cambanis A et al. A one-day method for the diagnosis of pulmonary tuberculosis in rural Ethiopia. *International Journal of Tuberculosis and Lung Disease*, 2006, 10(2):230–323.

102. Minion J, Sohn H, Pai M. Light-emitting diode technologies for TB diagnosis: what is on the market? *Expert Review of Medical Devices*, 2009, 6(4):341–345.

103. Ramsay A et al. New policies, new technologies: modelling the potential for improved smear microscopy services in Malawi. *PLoS One*, 2009, 4(11):e7760.

104. Breslauer DN et al. Mobile phone based clinical microscopy for global health applications. *PLoS One*, 2009, 4(7):e6320.

105. Sadaphal P et al. Image processing techniques for identifying *Mycobacterium tuberculosis* in Ziehl–Neelsen stains. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(5):579–582.

106. Dinnes J et al. A systematic review of rapid diagnostic tests for the detection of tuberculosis infection. *International Journal of Health Technology Assessment*, 2007, 11(3):1–196.

107. Cruciani M et al. Meta-analysis of BACTEC MGIT 960 and BACTEC 460 TB, with or without solid media, for detection of mycobacteria. *Journal of Clinical Microbiology*, 2004, 42(5):2321–2325.

108. *The use of liquid medium for culture and DST*. Geneva, World Health Organization, 2007. Available from: http://www.who.int/tb/laboratory/policy_liquid_medium_for_culture_dst/en/

109. Martin A et al. Implementation of the thin layer agar method for diagnosis of smear-negative pulmonary tuberculosis in a setting with a high prevalence of human immunodeficiency virus infection in Homa Bay, Kenya. *Journal of Clinical Microbiology*, 2009, 47(8):2632–2634.

110. Shikama ML et al. Multicentre study of nitrate reductase assay for rapid detection of rifampicin-resistant *M. tuberculosis*. *International Journal of Tuberculosis and Lung Disease,* 2009, 13(3):377–380.

111. Palomino JC. Nonconventional and new methods in the diagnosis of tuberculosis: feasibility and applicability in the field. *European Respiratory Journal*, 2005, 26:339–350.

112. Bwanga F et al. Direct susceptibility testing for multi drug resistant tuberculosis: A meta-analysis. *BMC Infectious Diseases*, 2009, 9:67.

113. Palomino JC. Molecular detection, identification and drug resistance detection in Mycobacterium tuberculosis. *FEMS Immunology and Medical Microbiology*, 2009, 56:103–111.

114. *Non-commercial culture and drug-susceptibility testing methods for screening of patients at risk of multi-drug resistant tuberculosis. Policy statement*. Geneva, World Health Organization, 2010.

115. Centers for Disease Control and Prevention. Updated guidelines for the use of nucleic acid amplification tests in the diagnosis of tuberculosis. *Morbidity and Mortality Weekly Report*, 2009, 58:7–10.

116. Greco S et al. The current evidence on diagnostic accuracy of commercial based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis. *Thorax*, 2006, 61:783–790.

117. Ling DI et al. Commercial nucleic-acid amplification tests for diagnosis of pulmonary tuberculosis in respiratory specimens: meta-analysis and meta-regression. *PLoS One*, 2008, 3(2):e1536.

118. Pai M et al. Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis. *BMC Infectious Diseases*, 2004, 4:6.

119. Pai M et al. Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis. *Lancet Infectious Diseases*, 2003, 3:633–643.

120. Boehme CC et al. Operational feasibility of using loop-mediated isothermal amplification for diagnosis of pulmonary tuberculosis in microscopy centers of developing countries. *Journal of Clinical Microbiology*, 2007, 45(6):1936–1940.

121. Boehme CC et al. Feasibility,diagnostic accuracy,and effectiveness of decentralised use of the Xpert MTB/RF test for diagnosis of tuberculosis and multidrug resistance: a multicentre implementation study. *Lancet*, 2011, 377(9776):1495–1505.

122. Vadwai V et al. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *Journal of Clinical Microbiology*, 2011, 49(7):2540–2545.

123. *Roadmap for rolling out Xpert MTB/RIF for rapid diagnosis of TB and MDR-TB*. Geneva, World Health Organization, 2010.

124. Morgan M et al. A commercial line probe assay for the rapid detection of rifampicin resistance in *Mycobacterium tuberculosis*: a systematic review and meta-analysis. *BMC Infectious Diseases*, 2005, 5:62.

125. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis of multidrug-resistant tuberculosis: a meta-analysis. *European Respiratory Journal*, 2008, 32(5):1165–1174.

126. Hillemann D, Rusch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl assay for fluoroquinolone, amikacin-capreomycin, and ethambutol resistance testing of *Mycobacterium tuberculosis* strains and clinical specimens. *Journal of Clinical Microbiology*, 2009, 47:1767–1772.

127. *The use of molecular line probe assay for the detection of resistance to second-line antituberculosis drugs.* Geneva, World Health Organization, 2013.

128. Steingart K, Henry M, Laal S. A systematic review of commercial serological antibody detection tests for the diagnosis of extra-pulmonary tuberculosis. *Thorax*, 2007, 62:911–918.

129. Steingart K, Henry M, Laal S. Commercial serological antibody detection tests for the diagnosis of pulmonary tuberculosis: a systematic review. *PLoS Medicine*, 2007,4:e202.

130. *Laboratory-based evaluation of 19 commercially available rapid diagnostic tests for tuberculosis.* Geneva, World Health Organization, 2008.

131. *Commercial serological tests for diagnosis of tuberculosis. Policy statement.* Geneva, World Health Organization, 2011.

132. Steingart KR et al. Performance of purified antigens for serodiagnosis of pulmonary tuberculosis: a meta-analysis. *Clinical Vaccine and Immunology*, 2009, 16(2):260–276.

133. Boehme CC et al. Detection of mycobacterial lipoarabinomannan with an antigen-capture ELISA in unprocessed urine of Tanzanian patients with suspected tuberculosis. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 2005, 99:893–900.

134. Mutetwa R et al. Diagnostic accuracy of commercial urinary lipoarabinomannan detection in African tuberculosis suspects and patients. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:1253–1259.

135. Daley P et al. Blinded evaluation of commercial urinary lipoarabinomannan for active tuberculosis: a pilot study. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:989–995.

136. Lawn SD et al. Urine lipoarabinomannan assay for tuberculosis screening before antiretroviral therapy diagnostic yield and association with immune reconstitution disease. *AIDS*, 2009, 23(14):1875–1880.

137. Shah M et al. Diagnostic accuracy of a urine lipoarabinomannan test for tuberculosis in hospitalized patients in a high HIV prevalence setting. *Journal of Acquired Immune Deficiency Syndromes*, 2009, 52(2):145–151.

138. Farhat M et al. False-positive tuberculin skin tests: what is the absolute effect of BCG and non-tuberculous mycobacteria? *International Journal of Tuberculosis and Lung Disease*, 2006, 10:1192–1204.

139. Menzies D, Pai M, Comstock GW. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Annals of Internal Medicine*, 2007, 146:340–354.

140. Pai M, Zwerling A, Menzies D. T-cell based assays for the diagnosis of latent tuberculosis infection: an update. *Annals of Internal Medicine*, 2008, 149:177–184.

141. Pai M, Menzies D. Interferon-gamma release assays: what is their role in the diagnosis of active tuberculosis? *Clinical Infectious Diseases*, 2007, 44:74–77.

142. Lange C et al. Interferon-gamma release assays for the diagnosis of active tuberculosis: sensible or silly? *European Respiratory Journal*, 2009, 33(6):1250–1253.

143. Cattamanchi A et al. Interferon-gamma release assays for the diagnosis of latent tuberculosis infection in HIV-infected individuals: a systematic review and meta-analysis. *Journal of Acquired Immune Deficiency Syndromes*, 2011 Mar 1, 56(3):230–238.

144. Pai M. Guidelines on IGRAs: concordant or discordant? Invited talk presented at: *Second Global Symposium on IGRAs. Dubrovnik, Croatia, 1 June 2009.*

145. Pai M et al. T-cell assays for the diagnosis of latent tuberculosis infection: moving the research agenda forward. *Lancet Infectious Diseases*, 2007, 7:428–438.

146. Pai M. Spectrum of latent tuberculosis: existing tests cannot resolve underlying phenotypes. *Nature Reviews Microbiology*, 2010, 8(3):242.

147. Dheda K et al. T-cell interferon-gamma release assays for the rapid immunodiagnosis of tuberculosis: clinical utility in high-burden vs. low-burden settings. *Current Opinion in Pulmonary Medicine*, 2009, 15:188–200.

148. *Use of tuberculosis interferon-gamma release assays (IGRAs) in low- and middle-income countries: policy statement.* Geneva, World Health Organization, 2011.

149. Wu X et al. Recombinant early secreted antigen target 6 protein as a skin test antigen for the specific detection of *Mycobacterium tuberculosis* infection. *Clinical & Experimental Immunology*, 2008, 152:81–87.

150. Paris Meeting on Point-of-Care Test Specifications. Available from: http://www.msfaccess.org/content/paris-meeting-tb-point-care-test-specifications (accessed 6 October 2012).

151. Marais BJ et al. Childhood pulmonary tuberculosis: old wisdom and new challenges. *American Journal of Respiratory and Critical Care Medicine*, 2006, 173:1078–1090.

152. Marais BJ, Pai M. New approaches and emerging technologies in the diagnosis of childhood tuberculosis. *Pediatric Respiratory Reviews*, 2007, 8:124–133.

153. Updated recommendations on interferon gamma release assays for latent tuberculosis infection. An Advisory Committee Statement (ACS). *Canada Communicable Disease Report*, 2008, 34(ACS-6):1–13.

154. Mazurek GH et al. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *Recommendations and Reports: Morbidity and Mortality weekly report*, 2005, 54:49–55.

155. Hopewell PC et al. International standards for tuberculosis care. *Lancet Infectious Diseases*, 2006, 6:710–725.

156. Theron G et al. Evaluation of the Xpert(R) MTB/RIF assay for the diagnosis of pulmonary tuberculosis in a high HIV prevalence setting. *American Journal of Respiratory and Critical Care Medicine*, 2011,184(1):132–140.

157. Lawn SD et al. Screening for HIV-associated tuberculosis and rifampicin resistance before antiretroviral therapy using the Xpert MTB/RIF assay: a prospective study. *PLoS Medicine*, 2011, 8(7):e1001067.

158. Sohn H et al. TB diagnostic tests: how do we figure out their costs? *Expert Review of Anti-InfectiveTherapy*, 2009,7(6):723–733.

159. Dowdy DW, O'Brien MA, Bishai D. Cost-effectiveness of novel diagnostic tools for the diagnosis of tuberculosis. *International Journal Tuberculosis and Lung Disease*, 2008 Sep,12(9):1021–1029.

160. Jindani A, Dore CJ, Mitchison DA. Bactericidal and sterilizing activities of antituberculosis drugs during the first 14 days. *American Journal of Respiratory and Critical Care Medicine*, 2003, 167(10):1348–1354.

161. Ginsberg AM, Spigelman M. Challenges in tuberculosis drug research and development. *Nature Medicine*, 2007, 13(3):290–294.

162. Marais BJ, Schaaf HS, Donald PR. Pediatric TB: issues related to current and future treatment options. *Future Microbiology*, 2009, 4(6):661–675.

163. Ginsberg AM, Spigelman M. New drugs for tuberculosis. In: Raviglione M, ed. *Tuberculosis, 4th ed: the essentials*. New York, Informa Healthcare, 2009: 344–363.

164. Ginsberg AM. New drugs and clinical drug trials. In: Kaufmann SH, Helden P, eds. *Handbook of tuberculosis: clinics, diagnostics, therapy and epidemiology*. Weinheim, Wiley–VCH, 2008: 213–222.

165. Ma Z, Lienhardt C. Toward an optimized therapy for tuberculosis? Drugs in clinical trials and in preclinical development. *Clinics in Chest Medicine*, 2009, 30(4):755–768.

166. Ma Z, Ginsberg AM, Spigelman M. Anti-mycobacterium agents. In: Taylor J, Triggle D, eds. *Comprehensive medicinal chemistry II,* vol. 7. Oxford, Elsevier, 2006:699–730.

167. Grosset J. The sterilizing value of rifampicin and pyrazinamide in experimental short-course chemotherapy. *Bulletin of the International Union against Tuberculosis and Lung Disease*, 1978, 53(1):5–12.

168. Sirgel FA et al. The early bactericidal activities of rifampin and rifapentine in pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine,* 2005, 172(1):128–135.

169. Mitchison DA. Role of individual drugs in the chemotherapy of tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 2000, 4(9):796–806.

170. Rosenthal IM et al. Daily dosing of rifapentine cures tuberculosis in three months or less in the murine model. *PLoS Medicine*, 2007, 4(12):e344.

171. An international multicentre controlled clinical trial to evaluate high dose RIFApentine and a QUINolone in the treatment of pulmonary tuberculosis. [Current Controlled Trials ISRCTN identifer: ISRCTN44153044]. Available from: http://www.controlled-trials.com/ISRCTN44153044/rifaquin (accessed 10 November 2012).

172. Pharmacokinetics and pharmacodynamics of high versus standard dose rifampicin in patients with pulmonary tuberculosis (high RIF) [clinicaltrials.gov identifier: NCT00760149]. US National Institutes of Health, ClinicalTrials.gov. Available from: **http://clinicaltrials.gov/show/NCT00760149** (accessed 10 November 2012).

173. Study of daily rifapentine for pulmonary tuberculosis [clinicaltrials.gov identifier: NCT00814671]. US National Institutes of Health, ClinicalTrials.gov. Available from: **http://clinicaltrials.gov/ct2/show/NCT00814 671?term=NCT00814671&rank=1 (accessed 10 November 2012**).

174. TBTC study 29: Rifapentine during intensive phase tuberculosis (TB) treatment [clinicaltrials.gov identifier: NCT00694629]. US National Institutes of Health, ClinicalTrials.gov. Available from: **http:// clinicaltrials.gov/ct2/show/NCT00694629? term=NCT00694629&rank=1 (accessed 10 November 2012**).

175. Hu Y, Coates AR, Mitchison DA. Sterilizing activities of fluoroquinolones against rifampin-tolerant populations of *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy*, 2003, 47(2):653–657.

176. Lounis N et al. Effectiveness of once-weekly rifapentine and moxifloxacin regimens against *Mycobacterium tuberculosis* in mice. *Antimicrobial Agents and Chemotherapy*, 2001, 45:3482–3486.

177. Nuermberger EL et al. Moxifloxacin-containing regimen greatly reduces time to culture conversion in murine tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2004, 169(3):421-426.

178. Gosling RD et al. The bactericidal activity of moxifloxacin in patients with pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2003, 168(11):1342–1345.

179. Pletz MW et al. Early bactericidal activity of moxifloxacin in treatment of pulmonary tuberculosis: a prospective, randomized study. *Antimicrobial Agents and Chemotherapy*, 2004, 48(3):780–782.

180. Rustomjee R et al. A phase II study of the sterilising activities of ofloxacin, gatifloxacin and moxifloxacin in pulmonary tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(2):128–138.

181. Burman WJ et al. Moxifloxacin versus ethambutol in the first 2 months of treatment for pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2006, 174(3):331–338.

182. Conde MB et al. Moxifloxacin versus ethambutol in the initial treatment of tuberculosis: a double-blind, randomised, controlled phase II trial. *Lancet*, 2009, 373(9670):1183–1189.

183. Dorman SE et al. Substitution of moxifloxacin for isoniazid during intensive phase treatment of pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2009, 180(3):273–280.

184. Controlled comparison of two moxifloxacin containing treatment shortening regimens in pulmonary tuberculosis (REMoxTB) [Clinicaltrials.gov idenitifier: NCT00864383]. US National Institutes of Health, ClinicalTrials.gov. Available from: **http://clinicaltrials.gov/show/NCT00864383** (accessed 10 November 2012).

185. A controlled trial of a 4-month quinolone-containing regimen for the treatment of pulmonary tuberculosis [clinicaltrials.gov identifier: NCT00216385]. US National Institutes of Health, ClinicalTrials.gov. Available from: **http://clinicaltrials.gov/ct2/show/NCT00216385** (accessed 11 October 2012).

186. Rich ML et al. Representative drug susceptibility patterns for guiding design of retreatment regimens for MDR-TB. *International Journal of Tuberculosis and Lung Disease*, 2006, 10(3):290–296.

187. Yew WW, Leung CC. Management of multidrug-resistant tuberculosis: Update 2007. *Respirology*, 2008,13(1):21–46.

188. Kobaidze K, Salakaia A, Blumberg HM. Over the counter availability of antituberculosis drugs in Tbilisi, Georgia, in the setting of a high prevalence of MDR-TB. *Interdisciplinary Perspectives on Infectious Diseases*, 2009, 2009:513609).

189. Vinh DC, Rubinstein E. Linezolid: a review of safety and tolerability. *Journal of Infection*, 2009, 59(Suppl 1):S59–74.

190. Schecter GF et al. Linezolid in the treatment of multidrug-resistant tuberculosis. *Clinical Infectious Diseases*, 2010, 50(1):49–55.

191. Migliori GB et al. A retrospective TBNET assessment of linezolid safety, tolerability and efficacy in multidrug-resistant tuberculosis. *European Respiratory Journal*, 2009, 34:387–393.

192. Williams KN et al. Addition of PNU-100480 to first-line drugs shortens the time needed to cure murine tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2009, 180(4):371–376.

193. Dietze R et al. Early and extended early bactericidial activtiy of linezolid in pulmonary tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2008, 178(11):1180–1185.

194. A placebo-controlled, phase 2 trial to evaluate OPC 67683 in patients with pulmonary sputum culture-positive, multidrug-resistant tuberculosis (TB) [Clinicaltrials.gov idenitifier: NCT00685360]. US National Institutes of Health, ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT00685 360?term=NCT00685360&rank=1 (accessed 10 November 2012).

195. Gler MT et al. Delamanid for multidrug-resistant pulmonary tuberculosis. *New England Journal of Medicine*, 2012, 366(23): 2151–2160.

196. Ginsberg AM et al. Safety, tolerability, and pharmacokinetics of PA-824 in healthy subjects. *Antimicrobial Agents and Chemotherapy*, 2012, 53(9):3720–3725.

197. Ginsberg AM et al. Assessment of the effects of the nitroimidazo-oxazine PA-824 on renal function in healthy subjects. *Antimicrobial Agents Chemotherapy*, 2009, 53(9):3726–3733.

198. Diacon AH et al. Early bactericidal activity and pharmacokinetics of PA-824 in smear-positive tuberculosis patients. *Antimicrobial Agents Chemotherapy*, 2010, 54(8):3402–3407.

199. Diacon AH et al. 14-day bactericidal activity of PA-824, bedaquiline, pyrazinamide, and moxifloxacin combinations: a randomised trial. *Lancet*, 2012, 380(9846):986–993.

200. Andries K et al. A diarylquinoline drug active on the ATP synthase of *Mycobacterium tuberculosis*. *Science*, 2005, 307:223–227.

201. Koul A et al. Diarylquinolines target subunit c of mycobacterial ATP synthase. *Nature Chemical Biology*, 2012, 3(6):323–324.

202. Koul A et al. Diarylquinolines are bactericidal for dormant mycobacteria as a result of disturbed ATP homeostasis. *The Journal of Biological Chemistry*, 2008, 283(37):25273–25280.

203. Diacon AH et al. The diarylquinoline TMC207 for multidrug resistant tuberculsosis. *New England Journal of Medicine*, 2009, 360(23):2397–2405.

204. McNeeley DF et.al. TMC207 versus placebo plus OBT for the treatment of MDR-TB: a prospective clinical trial.In: Abstract book of the Forty-first World Conference on Lung Health of the International Union against Tuberculosis and Lung Disease. *International Journal of Tuberculosis and Lung Disease*, 2010, 14(11 Suppl 2):S4.

205. Boshoff HI et al. The transcriptional responses of *M. tuberculosis* to inhibitors of metabolism: novel insights into drug mechanisms of action. *Journal of Biological Chemistry*, 2004, 279:4017–440184.

206. Thalan K et al. SQ109 targets MmpL3, a membrane transporter of trehalose monomycolate involved in mycolic acid donation to the cell wall core of *Mycobacterium tuberculosis*. *Antimicrobial Agents and Chemotherapy*, 2012, 56(4):1797–1809.

207. Dose escalation study of SQ109 in healthy adult volunteers [ClinicalTrials.gov identifier: NCT00866190]. US National Institutes of Health, ClinicalTrials.gov. Available from: http://www.clinicaltrials.gov/ct2/show/NCT00866190?term=NCT00866190&rank=1 (accessed 23 February 2013).

208. Early bactericidal activity of SQ109 in adult subjects with pulmonary tuberculosis (SQ109 EBA). [Clinicaltrials.gov idenitifier: NCT01218217]. US National Institutes of Health, ClinicalTrials.gov. Available from: http://clinicaltrials.gov/ct2/show/NCT01218217 (accessed February 2013)

209. Sizemore C, Heilman C. The changing face of biomedical research in tuberculosis. *Clinical Microbiology Newsletter*, 2008, 30:22.

210. *Tuberculosis research and development: 2009 report on tuberculosis research funding trends, 2005–2008.* New York, Treatment Action Group, 2009.

211. *WHO three I's meeting. Report of a joint WHO HIV/AIDS and TB Department meeting.* Geneva, World Health Organization, 2008.

212. *Global tuberculosis control 2009, epidemiology, strategy, financing.* Geneva, World Health Organization, 2009 (WHO/HTM/TB/2009.426).

213. Desormeaux J et al. Widespread HIV counseling and testing linked to a community-based tuberculosis control program in a high-risk population. *Bulletin of the Pan American Health Organization*, 1996, 30:1–8.

214. Espinal MA et al. Screening for active tuberculosis in HIV testing centre. *Lancet*, 1995, 345:890–893.

215. Mugisha B et al. Tuberculosis case finding and preventive therapy in an HIV voluntary counseling and testing center in Uganda. *International Journal of Tuberculosis and Lung Disease*, 2006, 10(7):761–767.

216. Shah S et al. Intensified tuberculosis case finding among HIV-Infected persons from a voluntary counseling and testing center in Addis Ababa, Ethiopia. *Journal of Acquired Immune Deficiency Syndromes*, 2009, 50:537–545.

217. Report of a "lessons learned" workshop of the sixth *ProTEST pilot projects in Malawi, South Africa and Zambia.* Geneva, World Health Organization; 2004 (WHO/HTM/TB/2004.336).

218. Chheng P et al. Pulmonary tuberculosis among patients visiting a voluntary confidential counseling and testing center, Cambodia. *International Journal of Tuberculosis and Lung Disease*, 2008, 12:54–62.

219. Shetty PV et al. Cross-referral between voluntary HIV counselling and testing centres and TB services, Maharashtra, India, 2003–2004. *International Journal of Tuberculosis and Lung Disease*, 2008, 12:26–31.

220. Kali PB et al. Combining PMTCT with active case finding for tuberculosis. *Journal of Acquired Immune Deficiency Syndromes*, 2006, 42:379–381.

221. Gasana M et al. Integrating tuberculosis and HIV care in rural Rwanda. *International Journal of Tuberculosis and Lung Disease*, 2008, 12:39–43.

222. Lawn SD et al. Screening for active tuberculosis among patients accessing antiretroviral therapy in sub-Saharan Africa. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:1186–1187.

223. McIntyre CR et al. Impact of tuberculosis control measures and crowding on the incidence of tuberculous infection in Maryland prisons. *Clinical Infectious Diseases*, 1997, 24:1060–1067.

224. Assefzadeh M, Barghi RG, Shahidi SS. Tuberculosis case--finding and treatment in the central prison of Qazvin province, Islamic Republic of Iran. *Eastern Mediterranean Health Journal*, 2009, 15:258–263.

225. Rao NA. Prevalence of pulmonary tuberculosis in Karachi central prison. *Journal of the Pakistan Medical Association*, 2004, 54:413–415.

226. Banda HT et al. Prevalence of smear-positive pulmonary tuberculosis among prisoners in Malawi: a national survey. *International Journal of Tuberculosis and Lung Disease*, 2009, 13(12):1557–1579.

227. Jittimanee SX et al. A prevalence survey for smear-positive tuberculosis in Thai prisons. *International Journal of Tuberculosis and Lung Disease*, 2007, 11:556–561.

228. Sanchez A et al. Screening for tuberculosis on admission to highly endemic prisons? The case of Rio de Janeiro State prisons. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:1247–1252.

229. Lewis JJ et al. HIV infection does not affect active case finding of tuberculosis in South African gold miners. *American Journal of Respiratory and Critical Care Medicine*, 2012, 180:1271–1278.

230. Cain KP et al. Tuberculosis among foreign-born persons in the United States: achieving tuberculosis elimination. *American Journal of Respiratory and Critical Care Medicine*, 2007, 175(1):75–79.

231. Claessens NJ et al. Screening childhood contacts of patients with smear-positive pulmonary tuberculosis in Malawi. *International Journal of Tuberculosis and Lung Disease*, 2002, 6(4):362–364.

232. Joshi R et al. Prevalence of abnormal radiological findings in health care workers with latent tuberculosis infection and correlations with T cell immune response. *PLoS One*, 2007, 2:e805.

233. Lien LT et al. Prevalence and risk factors for tuberculosis infection among hospital workers in Hanoi, Viet Nam. *PLoS One*, 2009, 4:e6798.

234. Asch S et al. Tuberculosis in homeless patients: potential for case finding in public emergency departments. *Annals of Internal Medicine*, 1998, 32:144–147.

235. Chaves AD, Robins AB, Abeles H. Tuberculosis case finding among homeless men in New York City. *American Review of Respiratory Disease*, 1961, 84:900–901.

236. Bothamley GH et al. Active case finding of tuberculosis in Europe: a Tuberculosis Network European Trials Group (TBNET) survey. *European Respiratory Journal*, 2008, 32:1023–1030.

237. den Boon S et al. An evaluation of symptom and chest radiographic screening in tuberculosis prevalence surveys. *International Journal of Tuberculosis and Lung Disease*, 2006, 10:876–882.

238. Sekandi JN et al. Active case finding of undetected tuberculosis among chronic coughers in a slum setting in Kampala, Uganda. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:508–513.

239. Shargie EB, Morkve O, Lindtjorn B. Tuberculosis case-finding through a village outreach programme in a rural setting in southern Ethiopia: community randomized trial. *Bulletin of the World Health Organization*, 2006, 84:112–119.

240. Yimer S et al. Evaluating an active case-finding strategy to identify smear-positive tuberculosis in rural Ethiopia. *International Journal of Tuberculosis and Lung Disease*, 2009, 13:1399–1404.

241. Ayles H et.al. Prevalence of tuberculosis, HIV and respiratory symptoms in two Zambian communities: implications for tuberculosis control in the era of HIV. *PLoS One*, 2009, 4:e5602.

242. Wood R et al. Undiagnosed tuberculosis in a community with high HIV prevalence: implications for tuberculosis control. *American Journal of Respiratory and Critical Care Medicine*, 2007, 175:87–93.

243. Mtei L et al. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. *Clinical Infectious Diseases*, 2005, 40:1500–1507.

244. Escombe AR et al. The infectiousness of tuberculosis patients coinfected with HIV. *PLoS Medicine*, 2008, 5(9):e188.

245. Miller A et al. Controlled trial of active tuberculosis case finding in a Brazilian favela. *International Journal of Tuberculosis and Lung Disease*, 2010, 14(6):720–726.

246.   Ayles HM et al. ZAMSTAR, The Zambia South Africa TB and HIV Reduction Study: design of a 2 x 2 factorial community randomized trial. *Trials*, 2008, 9:63.

247.   Ayles H et al. Effect of household and community interventions on the burden of tuberculosis in Southern Africa: the ZAMSTAR cluster-randomised trial. *Lancet,* 2013, in press.

248.   *Guidelines for intensified tuberculosis case-finding and isoniazid preventive therapy for people living with HIV in resource-constrained settings*. Geneva, World Health Organization, 2011.

249.   Getahun H, Raviglione M. Active case-finding for TB in the community: time to act. *Lancet*, 2010, 376(9748):1205–1206.

250.   Cain KP et al. An algorithm for tuberculosis screening and diagnosis in people with HIV. *New England Journal of Medicine*, 2010, 362:707–716.

251.   *WHO policy on TB infection control in health-care facilities, congregate settings and households*. Geneva, World Health Organization, 2009 (WHO/HTM/TB/2009.419).

252.   Menzies D, Joshi R, Pai M. Risk of tuberculosis infection and disease associated with work in health care settings. *International Journal of Tuberculosis and Lung Disease*, 2007, 11(6):593–605.

253.   Joshi R et al. Tuberculosis among health-care workers in low- and middle-income countries: a systematic review. *PLoS Medicine*, 2006, 3:e494.

254.   Nardell EA. The role of ventilation in preventing nosocomial transmission of tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 1998, 2(9 Suppl 1):S110–117.

255.   Nardell EA. Environmental infection control of tuberculosis.Review. *Seminars in Respiratory Infections*, 2003, 18(4):307–319.

256.   Escombe AR et.al. Natural ventilation for the prevention of airborne contagion. *PLoS Medicine*, 2007, 4:e68.

257.   Escombe AR et.al. Upper-room ultraviolet light and negative air ionization to prevent tuberculosis transmission. *PLoS Medicine*, 2009, 6:e43.

258.   Fennelly KP. The role of masks in preventing nosocomial transmission of tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 1998, 2(9 Suppl):S103–109.

259.   Harries AD et al. Preventing tuberculosis among health workers in Malawi. *Bulletin of the World Health Organization*, 2002, 80:526–531.

260.   Yanai H et al Risk of *Mycobacterium tuberculosis* infection and disease among health care workers, Chiang Rai, Thailand. *International Journal of Tuberculosis and Lung Disease*, 2003, 7:36–45.

261.   Roth VR et.al. A multicenter evaluation of tuberculin skin test positivity and conversion among health care workers in Brazilian hospitals. *International Journal of Tuberculosis and Lung Disease*, 2005, 9:1335–1342.

262.   da Costa PA et al. Administrative measures for preventing *Mycobacterium tuberculosis* infection among healthcare workers in a teaching hospital in Rio de Janeiro, Brazil. *Journal of Hospital Infection*, 2009, 72(1):57–64.

263.   Biscotto CR et al. Evaluation of N95 respirator use as a tuberculosis control measure in a resource-limited setting. *International Journal of Tuberculosis and Lung Disease*, 2009, 9(5):545–549.

264.   Chaisson RE, Harrington M. How research can help control tuberculosis. *International Journal of Tuberculosis and Lung Disease*, 2009, 13(5):558–568.

265. Sonnenberg P et al. How soon after infection with HIV does the risk of tuberculosis start to increase? A retrospective cohort study in South African gold miners. *Journal of Infectious Diseases*, 2005, 191(2):150–158.

266. Diedrich CR, Flynn JL. HIV/*M. tuberculosis* co-infection immunology: How does HIV exacerbate TB? *Infection and Immunity*, 2011, 79(4):1407–1417.

267. Lee MP et al. Clinical manifestations of tuberculosis in HIV-infected patients. *Respirology*, 2000, 5(4):423–426.

268. Poprawski D, Pitisuttitum P, Tansuphasawadikul S. Clinical presentations and outcomes of TB among HIV-positive patients. *The Southeastern Asian Journal of Tropical Medicine and Public Health*, 2000, 31(Suppl 1):140–142.

269. Goletti D et al. Effect of Mycobacterium tuberculosis on HIV replication. Role of immune activation. *Journal of Immunology*, 1996, 157(3):1271–1278.

270. Harries AD, Zachariah R, Lawn SD. Providing HIV care for co-infected tuberculosis patients: a perspective from sub-Saharan Africa. *International Journal of Tuberculosis and Lung Disease*, 2009, 13(1):6–16.

271. Lawn SD, Edwards DJ, Wood R. Concurrent drug therapy for tuberculosis and HIV infection in resource-limited settings: present status and future prospects. *Future HIV Therapy*, 2007, 1(4):387–398.

272. Vernon A et al. Acquired rifamycin monoresistance in patients with HIV-related tuberculosis treated with once-weekly rifapentine and isoniazid. *Lancet*, 1999, 353(9167):1843–1847.

273. Nettles RE et al. Risk factors for relapse and acquired rifamycin resistance after directly observed tuberculosis treatment: a comparison by HIV serostatus and rifamycin use. *Clinical Infectious Diseases*, 2004, 38(5):731–736.

274. *Treatment of tuberculosis: guidelines*, 4th ed. Geneva, World Health Organization, 2010.

275. Churchyard GJ et al. Efficacy of secondary isoniazid preventive therapy among HIV-infected Southern Africans: time to change policy? *AIDS*, 2003, 17(14):2063–2070.

276. Mukadi YD, Maher D, Harries AD. Tuberculosis case fatality rates in high HIV prevalence populations in sub-Saharan Africa. *AIDS*, 2001, 15(2):143–152.

277. Lawn SD, Kranzer K, Wood R. Antiretroviral herapy for control of the HIV-associated tuberculosis epidemic in resource-limited settings. *Clinics in Chest Medicine*, 2009, 30(4):685–699.

278. *Antiretroviral therapy for HIV infection in adults and adolescents. Recommendations for a public health approach: 2010 revision*. Geneva, World Health Organization, 2010.

279. Abdool Karim SS et al. Integration of antiretroviral therapy with tuberculosis treatment. *New England Journal of Medicine*, 2011, 365(16):1492–1501.

280. Havlir DV et al. Timing of antiretroviral therapy for HIV-1 infection and tuberculosis. *New England Journal of Medicine*, 2011, 365(16):1482–1491.

281. Blanc FX et al. Earlier versus later start of antiretroviral therapy in HIV-infected adults with tuberculosis. *New England Journal of Medicine*, 2011, 365(16):1471–1481.

282. Panel on Antiretroviral Guidelines for Adults and Adolescents. *Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents*. Department of Health andHuman Services. Available at: http://aidsinfo.nih.gov/ContentFiles/AdultandAdolescentGL.pdf (accessed 15 March 2013).

283.   McIlleron H et al. Complications of antiretroviral therapy in patients with tuberculosis: drug interactions, toxicity, and immune reconstitution inflammatory syndrome. *Journal of Infectious Diseases*, 2007, 196(Suppl 1):S63–75.

284.   Boulle A et al. Outcomes of nevirapine- and efavirenz-based antiretroviral therapy when coadministered with rifampicin-based antitubercular therapy. *Journal of the American Medical Association*, 2008, 300(5):530–539.

285.   Abdool Karim SS et al. Timing of initiation of antiretroviral drugs during tuberculosis therapy. *New England Journal of Medicine*, 2010, 362(8):697–706.

286.   *Guidelines for the programmatic management of drug-resistant tuberculosis.* 2011 update. Geneva, World Health Organization, 2011 (WHO/HTM/ TB/2011.6).

287.   Orenstein EW et al. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. *Lancet Infectious Diseases*, 2009, 9(3):153–161.

288.   Scano F et al. Management of HIV-infected patients with MDR- and XDR-TB in resource-limited settings. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(12):1370–1375.

289.   Dheda K et al. Early treatment outcomes and HIV status of patients with extensively drug-resistant tuberculosis in South Africa: a retrospective cohort study. *Lancet*, 2010, 375(9728):1798–1807.

290.   Meintjes GA et al. Novel relationship between tuberculosis immune reconstitution inflammatory syndrome and antitubercular drug resistance. *Clinical Infectious Diseases*, 2009, 48(5):667–676.

291.   Badri M, Wilson D, Wood R. Effect of highly active antiretroviral therapy on incidence of tuberculosis in South Africa: a cohort study. *Lancet*, 2002, 359(9323):2059–2064.

292.   Lawn SD, Badri M, Wood R. Tuberculosis among HIV-infected patients receiving HAART: long term incidence and risk factors in a South African cohort. *AIDS*, 2005, 19(18):2109–2116.

293.   Lawn SD et al. Burden of tuberculosis in an antiretroviral treatment programme in sub-Saharan Africa: impact on treatment outcomes and implications for tuberculosis control. *AIDS*, 2006, 20(12):1605–1612.

294.   Lawn SD et al. Short-term and long-term risk of tuberculosis associated with CD4 cell recovery during antiretroviral therapy in South Africa. *AIDS*, 2009, 23(13):1717–1725.

295.   Lawn SD, Bekker LG, Wood R. How effectively does HAART restore immune responses to *Mycobacterium tuberculosis*? Implications for tuberculosis control. *AIDS*, 2005, 19(11):1113–1124.

296.   Girardi E et al. Incidence of tuberculosis among HIV-infected patients receiving highly active antiretroviral therapy in Europe and North America. *Clinical Infectious Diseases*, 2005, 41(12):1772–1782.

297.   Williams BG, Dye C. Antiretroviral drugs for tuberculosis control in the era of HIV/AIDS. *Science*, 2003, 301(5639):1535–1537.

298.   Pacheco AG et al. AIDS-related tuberculosis in Rio de Janeiro, Brazil. *PLoS One*, 2008, 3(9):e3132.

299.   Granich RM et al. Universal voluntary HIV testing with immediate antiretroviral therapy as a strategy for elimination of HIV transmission: a mathematical model. *Lancet*, 2009, 373(9657):48–57.

300.   Smieja MJ et al. Isoniazid for preventing tuberculosis in non-HIV infected persons. *Cochrane Database of Systematic Reviews*, 2000, 2:CD001363.

301.   Woldehanna S, Volmink J. Treatment of latent tuberculosis infection in HIV infected persons. *Cochrane Database of Systematic Reviews*, 2004, 1:CD000171.

302.   Zar HJ et al. Effect of isoniazid prophylaxis on mortality and incidence of tuberculosis in children with HIV: randomised controlled trial. *BMJ*, 2007, 334(7585):136.

303.   Quigley MA et al. Long-term effect of preventive therapy for tuberculosis in a cohort of HIV-infected Zambian adults. *AIDS*, 2001, 15(2):215–222.

304.   Johnson DL et al. Duration of efficacy of treatment of latent tuberculosis infection in HIV-infected adults. *AIDS*, 2001, 15(16):2137–2147.

305.   Casado JL et al. Risk factors for development of tuberculosis after isoniazid chemoprophylaxis in human immunodeficiency virus-infected patients. *Clinical Infectious Diseases*, 2002, 34(3):386–389.

306.   Samandari T et al. 6-month versus 36-month isoniazid preventive treatment for tuberculosis in adults with HIV infection in Botswana: a randomised, double-blind, placebo-controlled trial. *Lancet*, 2011, 377(9777):1588–1598.

307.   Martinson NA et al. Novel regimens for treating latent TB in HIV-infected adults in South Africa: a randomised clinical trial. Paper presented at: *Sixteenth Conference on Retroviruses and Opportunistic Infections, Montreal, Canada, 8-11 February 2009.* (Abstract 36bLB).

308.   Swaminathan S et al. Efficacy of a 6-month vs a 36-month regimen for prevention of TB in HIV-infected persons in India: a randomised clinical trial. Paper presented at: *Seventeenth Conference on Retroviruses and Opportunistic Infections, San Francisco, CA, 16-19 February 2010.* (Abstract 104LB).

309.   Fitzgerald DW et al. Effect of post-treatment isoniazid on prevention of recurrent tuberculosis in HIV-1-infected individuals: a randomised trial. *Lancet*, 2000, 356(9240):1470–1474.

310.   Haller L et al. Isoniazid plus sulphadoxine–pyrimethamine can reduce morbidity of HIV-positive patients treated for tuberculosis in Africa: a controlled clinical trial. *Chemotherapy*, 1999, 45(6):452–465.

311.   Currie CS. Cost, affordability and cost-effectiveness of strategies to control tuberculosis in countries with high HIV prevalence. *BMC Public Health*, 2005, 5:130.

312.   Currie CS et al. Tuberculosis epidemics driven by HIV: is prevention better than cure? *AIDS*, 2003, 17(17):2501–2508.

313.   Cohen T et al. Beneficial and perverse effects of isoniazid preventive therapy for latent tuberculosis infection in HIV-tuberculosis coinfected populations. *Proceedings of the National Academy of Sciences of the United States of America*, 2006, 103(18):7042–7047.

314.   Lawn SD et al. Complementary roles of antiretrovirals and isoniazid preventive therapy in the prevention of HIV-associated tuberculosis in resource-limited settings. *Lancet Infectious Diseases*, 2010, 10(7):489–498.

315.   Golub JE et al. The impact of antiretroviral therapy and isoniazid preventive therapy on tuberculosis incidence in HIV-infected patients in Rio de Janeiro, Brazil. *AIDS,* 2007, 21(11):1441–1448.

316.   Golub JE et al. Isoniazid preventive therapy, HAART and tuberculosis risk in HIV-infected adults in South Africa: a prospective cohort. *AIDS,* 2009, 23(5):631–636.

317.   *The Stop TB Strategy: building on and enhancing DOTS to meet the TB-related Millennium Development Goals.* Geneva, World Health Organization, 2006 (WHO/HTM/ STB/2006.368).

318. *Stop TB Policy Paper: contributing to health system strengthening: guiding principles for national tuberculosis control programmes*. Geneva, World Health Organization, 2008 (WHO/HTM/TB/2008.400).

319. Van Deun A et al. Performance and acceptability of the FluoLED Easy™ module for tuberculosis fluorescence microscopy. *International Journal of Tuberculosis and Lung Disease*, 2008, 12(9):1009–1014.

320. Marais BJ et al. Use of light-emitting diode fluorescence microscopy to detect acid-fast bacilli in sputum. *Clinical Infectious Diseases*, 2008, 47(2):203–207.

321. Botha E et al. Initial default from tuberculosis treatment: how often does it happen and what are the reasons? *International Journal of Tuberculosis and Lung Disease*, 2008, 12(7):820–823.

322. Sai BB et al. Initial default among diagnosed sputum smear-positive pulmonary tuberculosis patients in Andhra Pradesh, India. *International Journal of Tuberculosis Lung Disease*, 2008, 12(9):1055–1058.

323. Getahun H et al. Diagnosis of smear-negative pulmonary tuberculosis in people with HIV infection or AIDS in resource-constrained settings: informing urgent policy changes. *Lancet Infectious Diseases*, 2007, 369(9578):2042–2049.

324. Grant AD, Gothard P, Thwaites G. Managing drug-resistant tuberculosis. *BMJ*, 2008, 337:a1110.

325. Nunn P et al. Tuberculosis control in the era of HIV. *Nature Reviews. Immunology*, 2005, 5(10):819–826.

326. De Cock KM et al. Can antiretroviral therapy eliminate HIV transmission? *Lancet*, 2009, 373(9657):7–9.

327. Rusen ID. Tuberculosis retreatment: a topic whose time has come. *International Journal of Tuberculosis and Lung Disease*, 2009, 13(10):1192.

328. Menzies D et al. Standardized treatment of active tuberculosis in patients with previous treatment and/or with mono-resistance to isoniazid: a systematic review and meta-analysis. *PLoS Medicine,* 2009, 6(9):e1000150.

329. *Treatment of tuberculosis guidelines*. 4th edition. Geneva, World Health Organization, 2010.

330. Van Deun A et al. Short, highly effective, and inexpensive standardized treatment of multidrug-resistant tuberculosis. *American Journal of Respiratory and Critical Care Medicine*, 2010, 182(5):684–692.

331. *Women and health: today's evidence tomorrow's agenda*. Geneva, World Health Organization, 2009.

332. *Global tuberculosis control report 2012*. Geneva, World Health Organization, 2012.

333. *An international roadmap for tuberculosis research: towards a world free of tuberculosis*. World Health Organization, Geneva, 2011.

334. Nachega J, et.al. Tuberculosis active case-finding in a mother-to-child HIV transmission prevention programme in Soweto, South Africa. *AIDS* 2003;(17):1398-1400.

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