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Assessment & analysis of HIV/MTB epidemiology and diagnostics as a basis for evidence-based guideline development & priority setting in Europe

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Content

Summary

Tuberculosis (TB) and the Acquired Immunodeficiency Syndrome (AIDS) are the two leading deadly infectious diseases globally, and in combination represent a public health problem of particular complexity. Though highly different in history and pathogenesis (AIDS is a viral infection, while TB is caused by the bacterium *M. tuberculosis*; AIDS is recently emerging and TB very ancient) the two diseases exert considerable mutual interactions that require specific public health measures and targeted research initiatives. The aim of this thesis was to provide an overview of the epidemiology of TB and HIV/*M. tuberculosis* co-infection and current diagnostic standards in Europe, identify priority areas for future research activities, and communicate the results to relevant stakeholders.

To prepare an evidence-base for research priority setting, data collected by the World Health Organization (WHO) and the European Centre for Disease Prevention and Care (ECDC) were combined to a comprehensive overview of the epidemiology of TB, drug-resistant TB, and co-infection with HIV in all 27 EU member states. This data collection was complemented by an assessment of diagnostic standards, looking both at the suitability of different test methods to actually diagnose active TB or latent infection, and at the availability and frequency of use of the respective tests. Modern blood-based Interferon-γ-release-assays (IGRAs) have been recently established as a more specific alternative to the classical tuberculin skin test (TST) for the diagnosis of latent TB infection, while their value for the diagnosis of active TB was unclear. This question was addressed in a systematic review and meta-analysis, and information on current clinical practice in Europe and non-European high burden areas was collected via an electronic survey.

Following the baseline assessment, research priorities were identified in an international collaborative effort. This thesis summarizes the outcomes of a consensus procedure to identify research needs in the area of HIV/*M. tuberculosis* co-infection which was performed by experts from Europe and the high burden areas India, Russia, Latin America, and Sub-Saharan Africa. In an interdisciplinary and intersectoral approach, concrete suggestions for initiatives were defined and recommended to the European Commission. A funding analysis was performed to identify current funding gaps and to ensure that the suggestions for new initiatives were complementary to ongoing activities. Finally, to inform decision makers, raise further awareness for the issue of HIV/*M. tuberculosis* co-infection and advocate for

increased funding specifically for this area of research, a short film was produced which explains the severity of the problem, emphasizes the urgency of further research, and highlights the suggested focus topics.

Results and conclusions

The epidemiological overview illustrates that TB surveillance data in Europe are incomplete, and particularly scarce for co-infection with HIV. Those data that were available revealed a diverse distribution pattern with both high and low prevalence countries among the 27 member states, and hotspots scattered throughout. This suggests that generalized approaches for the whole of Europe may be oversimplistic, and emphasizes the need of improved disease monitoring to allow targeted planning of effective control measures and new research initiatives.

Diagnostic standards in Europe on the other hand are high, especially in comparison to most high prevalence regions, as confirmed by the results of the electronic survey. According to the collected information, IGRAs are widely available and routinely applied in most European countries. Their sensitivity is higher than that of the TST but not sufficient to recommend them as a single rule out test for active TB, as the meta-analysis data suggest. Also, specificity is too low to distinguish active disease from latent infection. These results have already been taken up in ECDC guidelines for the use of IGRAs, which may contribute to a further improvement and harmonization of diagnostic standards in Europe. The meta-analysis furthermore showed that, when applied to extransanguinous fluids, the T-SPOT.*TB* assay seems to be the best commercially available immunodiagnostic test to detect active TB, but more independent and carefully designed prospective studies are needed to confirm these results.

This is in line with the results of the consensus procedure which identified diagnostics as one of the main areas of interest, along with specific questions in basic research, clinical sciences, training and networking. While several of these recommendations have already been reflected in the latest EU calls, co-infection remains a clear gap and needs increased support. The produced short film has proven as a highly useful means to advocate for such funding on local, national and global level.

Zusammenfassung

Tuberkulose und AIDS sind die beiden tödlichsten Infektionskrankheiten weltweit und stellen in ihrer Kombination ein besonders komplexes Problem für das globale Gesundheitswesen dar. Die vielfältigen Wechselwirkungen der beiden Erkrankungen erfordern spezifische Kontrollmaßnahmen und gezielte Forschungsinitiativen. Ziel dieser Arbeit war es, einen Überblick über die Epidemiologie der TB und der Ko-Infektion mit HIV sowie über diagnostische Standards in Europa zu geben, auf dessen Basis Forschungsprioritäten zu definieren, und diese an relevante Interessensgruppen zu kommunizieren.

Als Grundlage für die evidenzbasierte Definition von Forschungsprioritäten wurde mit Hilfe von Daten der Weltgesundheitsorganisation (WHO) und des Europäischen Zentrums für die Prävention und die Kontrolle von Krankheiten (ECDC) ein umfassender und detaillierter Überblick über die Epidemiologie der TB, der resistenten TB und der Ko-Infektion mit HIV in allen 27 EU Mitgliedsstaaten zusammengestellt. Dieser wurde um Informationen zu diagnostischen Standards in der klinischen Praxis ergänzt. In den letzten Jahren haben sich IGRAs, immundiagnostische Bluttests, als Alternative zum klassische intrakutanen Tuberkulin-Hauttest für die spezifische Diagnose einer latenten Tuberkuloseinfektion etabliert. Ihre Eignung zum Nachweis der aktiven TB war jedoch unklar und wurde daher im Rahmen einer systematischen Metaanalyse untersucht. Zusätzlich wurden in einer elektronischen Umfrage Daten zur Verfügbarkeit und zur Häufigkeit der Anwendung der jeweiligen Tests in Europa und in den am meisten durch diese Erkrankungen betroffenen Regionen der Welt erhoben.

Darauf basierend wurden unter Beteiligung von Experten aus Europa und den Hochprävalenzregionen Indien, Russland, Lateinamerika und Subäquatorialafrika Forschungsprioritäten im Bereich HIV/*M. tuberculosis* Ko-Infektion definiert, die in der vorliegenden Arbeit zusammengefasst sind. In einem interdisziplinären, sektorübergreifenden Ansatz wurden konkrete Vorschläge für zukünftige Initiativen erarbeitet und als Empfehlungen an die Europäische Kommission weitergegeben. Mit Hilfe einer Fördermittelanalyse wurde die europäische Forschungslandschaft hinsichtlich Finanzierungslücken untersucht um sicherzustellen, dass die erarbeiteten Vorschläge komplementär zu bestehenden Initiativen sind. Um Entscheidungsträger gezielt zu informieren, wurde ein Kurzfilm produziert, der die Komplexität des Problems vermittelt und

die Notwendigkeit weiterer Forschungsarbeiten im Bereich der HIV/*M. tuberculosis* Ko-Infektion erläutert.

Ergebnisse und Schlussfolgerungen

Der epidemiologische Überblick macht deutlich, dass Datenerfassung in Europa insbesondere zur Ko-Infektion mit HIV unvollständig ist. Es zeigt sich ein unregelmäßiges Verteilungsmuster von Hoch-und Niedrigprävalenzländern sowie vereinzelten Brennpunkten. Dies legt nahe, dass generalisierte Ansätze für Europa als Ganzes zu unspezifisch sind, und unterstreicht die Notwendigkeit einer verbesserten Krankheitsüberwachung als Basis für gezielte Planungen effektiver Kontrollmaßnahmen und neuer Forschungsinitiativen.

Die diagnostischen Standards innerhalb der EU sind dagegen hoch, insbesondere im Vergleich zu den untersuchten Hochprävalenzgebieten. Die elektronische Umfrage belegt, dass IGRAs in den meisten der 27 EU-Mitgliedsstaaten verfügbar und Teil der klinischen Routine sind. Die Metaanalyse zeigt, dass ihre Sensitivität die des klassischen intrakutanen Tuberkulin-Hauttests übertrifft, jedoch nicht hoch genug ist, um sie als alleinigen Test für den Ausschluss aktiver TB zu empfehlen. Darüber hinaus bestätigt die Analyse, dass die Spezifität der IGRAs nicht ausreicht, um zwischen aktiver und latenter TB zu differenzieren. Diese Resultate sind bereits in die Erstellung europäischer Richtlinien zur Nutzung von IGRAs eingeflossen. Weiterhin legen die Daten nahe, dass der T-SPOT.*TB* Assay der beste kommerziell verfügbare immundiagnostische Test für den Nachweis einer aktiven TB durch die Untersuchung extransanguiner Körperflüssigkeiten zu sein scheint. Diese Ergebnisse sind jedoch in zusätzlichen, unabhängigen prospektiven Studien zu bestätigen.

Neben der Diagnostik wurden unter Beteiligung des internationalen Expertengremium auch spezifische Fragestellungen im Bereich der Grundlagenforschung und der klinischen Forschung als Prioritäten erarbeitet und festgelegt, sowie auf die Bedeutung von gezielter Aus- und Weiterbildung im Bereich der HIV/*M. tuberculosis* Ko-Infektion hingewiesen. Während einige der Empfehlungen in den jüngsten Ausschreibungsrunden der EU-Kommission bereits umgesetzt wurden, bleibt im Bereich der HIV/*M. tuberculosis* Ko-Infektion ein klarer Bedarf für weitere Forschungsinitiativen bestehen. Der produzierte Kurzfilm hat sich als probates Mittel erwiesen, um auf regionaler, nationaler und globaler Ebene für weitere Fördermittel zu werben.

1 Introduction

Tuberculosis (TB) has plagued mankind for thousands of years and, though curable in principle, remains the most deadly infectious disease, with the estimated total number of incident cases of TB worldwide rising to 9.4 million in 2009 - more than at any other time in history (WORLD HEALTH ORGANIZATION, 2003; HERSHKOVITZ, 2008; LAWN, ZUMLA, 2011; WORLD HEALTH ORGANIZATION, 2011b). The infectious agent causing the disease, *Mycobacterium tuberculosis* (MTB), is responsible for more human deaths than any other single pathogen today (OTTENHOFF, KAUFMANN, 2012). Especially co-infection with the human immunodeficiency virus (HIV) has led to a major upsurge again and boosts the development and spread of drug resistant strains, which are currently posing a serious challenge particularly in Europe (WELLS et al., 2007; GANDHI et al., 2010).

1.1 The history of tuberculosis in Europe

The oldest evidence for MTB infection in humans so far was discovered in 2008 in human remains from 9,000 years ago in a settlement in the eastern Mediterranean (HERSHKOVITZ, 2008). As one of the earliest medical documentations, Hippocrates in 400 BC described *phtisis* as the most common cause of illness of his times, stating that the disease was affecting primarily young people between 18 and 35 of age, with a fatal outcome in the vast majority of cases (HIPPOCRATES, 400 BC).

A massive TB epidemic in Europe, known as the Great White Plague, started in the 17th century, and lasted for over 200 years. In peak times, TB case rates in London had reached 1,000 per 100,000 population per year, making it the principal cause of death by 1650. Once an individual was diagnosed with TB, death was considered inevitable. The high population density as well as poor sanitary conditions created an environment in which the disease could perfectly flourish. In the early 19th century, TB, often also referred to as *Consumption,* still accounted for up to 25% of deaths in Europe (LAWN, ZUMLA, 2011) but was now regarded as a 'romantic' disease and popularized as the disease of artists, with Frédéric Chopin, Paul Gauguin or Honoré de Balzac being just a few of many famous examples of the time.

The first to discover the pathogen causing TB was the German scientist and later Nobel Laureate Robert Koch, who presented his finding on March 24, 1882 at the Physiological

Society of Berlin. This day has since been known as World TB Day. Koch published his findings shortly afterwards, for the first time establishing a direct relationship between a causative microbe and an infectious disease. In his article entitled 'Die Ätiologie der Tuberkulose', Koch demonstrated that the bacterium he had identified was the single causative agent for all known forms of TB (KOCH, 1884).

In 1895, Wilhelm Roentgen discovered the X-ray, providing the first reliable tool for diagnosis and monitoring of disease progression. A few years later in 1908, tuberculin, a purified protein derivative (PPD) of the bacteria developed by Koch in 1890 - originally in search for an effective means of immunization - was shown by Felix Mendel and Charles Mantoux to be an effective intradermal test for diagnosing TB. The Mantoux test or Tuberculin Skin Test (TST) is still standard diagnostic practice throughout the world today (LANGE, SESTER, 2012).

With improving living standards (hygiene, better nutrition, improved housing conditions) early in the 20th century, the death toll from TB finally began to fall. Bacillus Calmette-Guérin (BCG) was described as a TB vaccine by Albert Calmette, director of the Institut Pasteur in Lille, and his colleague Camille Guérin in 1906, and entered into clinics in 1921. In 1943, the first antibiotic, streptomycin, was discovered by Albert Schatz, Elizabeth Bugie, and Selman Waksman in the US, and described as the first effective anti-TB drug a year later. Thus, by the second half of the 20th century, human TB was considered controllable, and its elimination just a matter of time (KESHAVJEE, FARMER, 2012).

Consequently, research and development (R&D) activities began to fade in the second half of the 19th century. As TB is a chronic disease, control failures do not become apparent immediately, though, but surface only much later (KAUFMANN, PARIDA, 2007). Today, significant setbacks clearly show that both scientists and clinicians had been falsely satisfied with the early achievements: i) BCG, still the only approved vaccine against TB to date, turned out to be not sufficiently effective. Despite the relative efficacy of BCG in infants, it fails to prevent pulmonary TB in adolescents (OTTENHOFF, KAUFMANN, 2012); ii) Development of (multi-) resistance against anti TB-drugs, as seen already in the early days of TB chemotherapy, has been massively increasing in the last years (NACHEGA, CHAISSON, 2003); iii) The global AIDS pandemic has caused an explosive increase in TB incidence (CORBETT et al., 2003; WELLS et al., 2007).

Consequently, TB is escalating again, and some aspects hit Europe particularly hard. According to estimates of the World Health Organization (WHO), two billion people are infected with MTB globally, i.e. one third of the world's population, representing an immense reservoir of the infectious agent. Thus, intensified efforts and concerted action are needed to tackle the global threat.

1.2 The pathogen

MTB is an intracellular pathogen that can infect several animal species, but human beings are the principal hosts. The straight or slightly curved MTB rods are ranging in size from 0.3 to 0.6 by 0.5 to 4.0 micrometers, occurring singly and in occasional threads (Figure 1). MTB is an aerobic, acid-fast and acid-alcohol-fast, gram-positive, non-motile, non-encapsulated bacillus which grows most successfully in tissues with high oxygen content such as the lungs. Even under ideal conditions, however, growth is extremely slow. MTB divides every 15 - 20h, while other bacteria usually divide in less than one hour (e.g. E. coli every 20 minutes) (WAYNE, KUBICA, 1986). This slow replication rate of MTB results in a particularly long time of several weeks to diagnosis in culture, and is also causal for the need of an unusually long duration of drug therapy, both for the treatment of disease as well as for preventive chemotherapy.

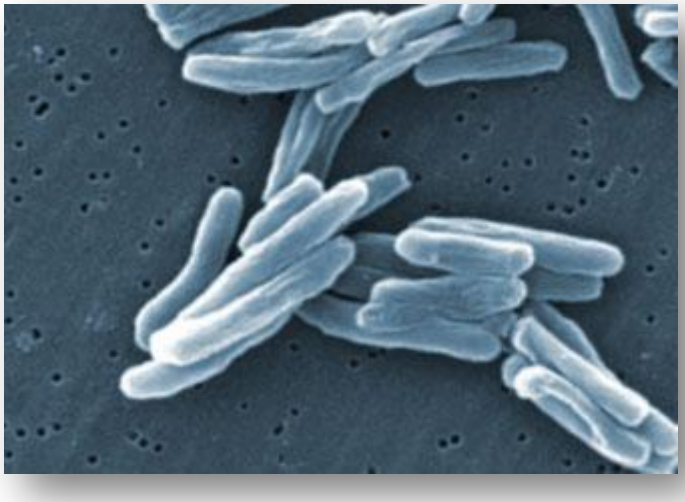


Figure 1. Mycobacterium tuberculosis. Source: CDC, 2011.

The bacterium belongs to the Mycobacterium Tuberculosis Complex (MTC), which groups mycobacteria that cause human and/or animal TB (as opposed to non-tuberculous mycobacteria, NTM) and is comprised of the classical species *M. tuberculosis*, *M. africanum*,

M. microti, and *M. bovis* (BROSCH et al., 2002), as well as newer additions *M. caprae* (ARANAZ et al., 2003), *M. pinnipedii* (COUSINS et al., 2003), and *M. canetti* (VAN SOOLINGEN et al., 1997).

M. africanum is globally not widespread, but it represents up to 60% of clinical strains from patients with pulmonary TB, particularly in Western African countries (NIEMANN et al., 2004). *M. microti* causes TB mainly in small rodents like voles, and only few cases of infections in humans have been described (XAVIER EMMANUEL et al., 2007). *M. bovis* can cause TB in various domestic or wild animals like cattle or goats (WAYNE, KUBICA, 1986), but can also infect humans via milk products. The introduction of pasteurization, however, has largely eliminated TB caused by *M. bovis* as a public health problem in humans (NIEMANN et al., 2000). *M. caprae* has been isolated from cattle, wild boar and pigs in different European countries (ARANAZ et al., 2003) and was recently shown to cause tuberculous meningitis also in humans (HANSEN et al., 2012), while *M. pinipedii* seems to be restricted to seals (COUSINS et al., 2003). *M. canetti* has been isolated from single human patients but seems very rare (VAN SOOLINGEN et al., 1997).

1.3 Transmission, infection, and immune response

TB may be the only communicable disease with obligate airborne transmission (ROY, MILTON, 2004). The pathogen is transmitted by aerosols which are produced by individuals with active TB. Infectious bacilli can be aerosolized by any respiratory manoeuvre, i.e. talking, singing, coughing, or sneezing. The larger the physical force, the higher the number of exhaled droplets and the smaller their size, and the quicker the tubercle bacilli evaporate into droplet nuclei - a form in which they can remain airborne for several hours. Transmission occurs when a new host inhales MTB, and the bacilli reach the alveoli of the lungs. Estimates of the minimum infectious dose range from a single bacterium upward, and the risk of infection is closely linked with time and frequency of exposure as well as age and immune status of the exposed individual (ERKENS et al., 2010; RUSSELL et al., 2010; OTTENHOFF, KAUFMANN, 2012). Uptake of MTB can result in different clinical outcomes, ranging from the complete absence of clinical or laboratory evidence of infection, to detectable infection with no signs of clinically active disease, to active TB (WALZL et al., 2011). The disease most commonly affects the lungs (‘pulmonary TB’) but can occur in almost any anatomical site or as disseminated disease.

The actual outcome of exposure depends on the ability of the host's innate and adaptive immune system to eradicate or control the bacterium. After inhalation into the lungs of a new host, the bacilli are phagocytosed by various cell types, including alveolar macrophages, interstitial macrophages, local dendritic cells, and likely also epithelial cells, as part of the innate immune system (OTTENHOFF, KAUFMANN, 2012). As part of adaptive immunity, $CD4^+$ T-cells secrete interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α), which activate macrophages to destroy ingested mycobacteria (FLYNN et al., 1993). However, the onset of adaptive immune response mechanisms is delayed by several weeks after infection with MTB as compared to other infections (URDAHL et al., 2011). During that initial phase of uninhibited exponential replication, two types of MTB populations can develop: (i) dormant bacilli with very low metabolic activity that do not replicate but can persist in infected cells over very long periods of time, presenting a potential reservoir of bacteria that can resuscitate later; and (ii) actively replicating bacteria (OTTENHOFF, KAUFMANN, 2012).

1.4 Latency

For immunocompetent individuals, the annual risk of developing active clinical TB after inhalation of viable MTB bacteria is relatively low and decreases over time, with an estimated lifetime risk of about 10% (DYE, WILLIAMS, 2010). In turn, this means that in over 90% of infected individuals, the immune system can either eliminate the bacilli, or keep the infection sufficiently under control. Such a quiescent infection is often referred to as 'latent tuberculosis infection' (LTBI), and typically comes along with evidence of T-cell priming (VAN SOOLINGEN et al., 1997).

After an initial phase of bacterial growth, activated macrophages are able to sustain bacterial load at a relatively constant level. The infection passes over to a state of chronicity, with bacteria persisting enclosed in granulomas. Initially they are surrounded by an amorphous accumulation of macrophages, monocytes, and neutrophils. With the development of an acquired immune response and the recruitment of MTB-specific lymphocytes about 2 to 3 weeks after infection, the granuloma develops a stratified structure and the caseous core of the granuloma becomes hypoxic – a condition which in culture has been shown to induce non-replicative persistence of MTB (RUSSELL et al., 2010).

LTBI is symptom-free, and infected individuals are not contagious. They may carry viable MTB for years and decades without ever developing any signs of disease, or showing

macroscopic or histological evidence of TB (HERNANDEZ-PANDO et al., 2000). However, granulomas are not fixed inert structures; cells are constantly dying and replaced by new cells, and debris is being removed. If the immunological balance is disturbed, however, active replication may start again and can lead to subclinical or even clinically active infection (VAN SOOLINGEN et al., 1997; BARRY et al., 2009; MACK et al., 2009). In this stage, patients re-establish infectiousness and - as long as untreated - will spread the infection on average to 10 to 15 new people per year (WORLD HEALTH ORGANIZATION, 2002), i.e. roughly one person per month.

While the traditional separation between active disease and latent infection as two distinct binary states works well in clinical and public health contexts, it is recognized that this model does not provide a sound basis for immunologic studies (ERNST et al., 2012). Latest evidence suggests that LTBI likely includes a much broader spectrum of states, ranging from complete clearance of infection to subclinical active disease, and that a continuous spectrum of these states exists both in the same individual and between different individuals (BARRY et al., 2009; LAWN et al., 2011; LAWN, ZUMLA, 2011; WALZL et al., 2011).

1.5 TB diagnostics

In addition to the evaluation of a patient's medical history, clinical symptoms and chest X-ray, active TB is diagnosed by the detection of the pathogen. The most commonly used diagnostic test for TB globally, **sputum smear microscopy** through staining of acid-fast bacilli (AFB) as described by Robert Koch, is 125 years old and routinely misses 50% of all cases. In fact, only 44% of all new adult cases and 15-20% of childhood cases are identified by the presence of AFB in sputum smears (NEWTON et al., 2008; WORLD HEALTH ORGANIZATION, 2009).

The gold standard for the definite diagnosis of active TB is therefore still the detection of MTB by **culture methods**, which takes several weeks due to the slow replication rate of the pathogen. Furthermore, MTB culture requires a laboratory infrastructure that is not widely available in resource poor settings hence cannot be used nationwide in many of the high burden countries.

Two diagnostic methods are currently in use to support the diagnosis of LTBI: the tuberculin skin test (TST) and *in vitro* interferon-γ release assays (IGRAs). Both tests are immunological

methods that measure specific acquired immune responses (MACK et al., 2009; YOUNG et al., 2009), and are thus an indirect marker for past or current infection.

For the **Tuberculin skin test (TST)**, a standard dose of tuberculin-purified protein derivative (PPD) is injected intradermally into the volar surface of the forearm and is read 48 to 72 hours later. The immune response is assessed by measuring the diameter of induration in millimeters. Depending on specific risk factors, an induration of 5mm, 10mm, or 15mm represent a positive result (MACK et al., 2009). However, the test has several shortcomings. Besides the difficulty of standardized read-outs, it is disadvantageous that patients must return to their health-care provider to have their test evaluated. Also, contact with NTM or BCG vaccination can lead to false-positive TST results, as the PPD contains antigens which are also present in BCG and certain NTM.

In recent years, blood-based ***in vitro*** **interferon-γ release assays (IGRAs)** have been developed as alternatives to the TST. IGRAs are more specific than the TST, as they are based on the *in vitro* stimulation of peripheral blood T-cells which are specific for the MTB specific antigens early secretory antigenic target-6 (ESAT-6) and culture filtrate protein-10 (CFP-10) (ANDERSEN et al., 2000), i.e. false-positive results from contact with NTM or BCG vaccination can be avoided. The presence of reactive T-cells is assessed by the induction of interferon-γ (IFN-γ). Two IGRAs are currently commercially available: The QuantiFERON®-TB Gold in-tube assay (QFT-GIT) produced by Cellestis, an Australian company which was recently acquired by Qiagen, Germany, and the T-Spot®.TB assay, which was developed by the University of Oxford and is now produced and distributed by Oxford Immunotec Ltd., UK.

The QuantiFERON®-TB Gold in-tube test uses the enzyme-linked immunosorbent assay (ELISA) detection method. The assay consists of 3 blood collection tubes (nil, TB antigens ESAT-6, CFP-10 and TB7.7 dried to the wall of the tubes, and mitogen (phyto-hæmagglutinin, PHA) (Figure 2). IFN-γ is measured from the supernatants of whole blood after a stimulation time of 16-24 hours. Results are interpreted qualitatively as negative, positive or indeterminate according to the manufacturer's recommendations (Table 1).

Figure 2. **QuantiFERON®-TB Gold in-tube blood collection tubes.**

Table 1. **Interpretation criteria for the QuantiFERON-TB Gold In-Tube assay (QFT-GIT).**

result	IFN-γ concentration (International Units per ml, IU/ml)		
	MTB antigens	*nil*	*PHA nil*
positive	≥ 0.35 IU/ml and ≥ 25% over nil	≤ 8.0 IU/ml	any
negative	< 0.35 IU/ml or < 25% over nil	≤ 8.0 IU/ml	≥ 0.5 IU/ml
indeterminate	<0.35 IU/ml or < 25% over nil	≤ 8.0 IU/ml	< 0.5 IU/ml
	any	> 8.0 IU/ml	any

For the **T-Spot®.*TB*** test, which is based on the enzyme-linked immunosorbent spot (ELISPOT) assay, peripheral blood mononuclear cells (PBMC) are isolated through density gradient centrifugation, washed and counted. 250,000 PBMC are added to wells that are pre-coated with IFN-γ antibodies and stimulated for 16-20 hours by ESAT-6 and CFP-10 along with negative (nil) and positive controls (PHA). Activated T-cells release IFN-γ which is locally captured and detected by a secondary antibody to IFN-γ. Spots are counted (Figure 3), and results are qualitatively interpreted as negative, positive or indeterminate according to the manufacturer's recommendations (Table 2).

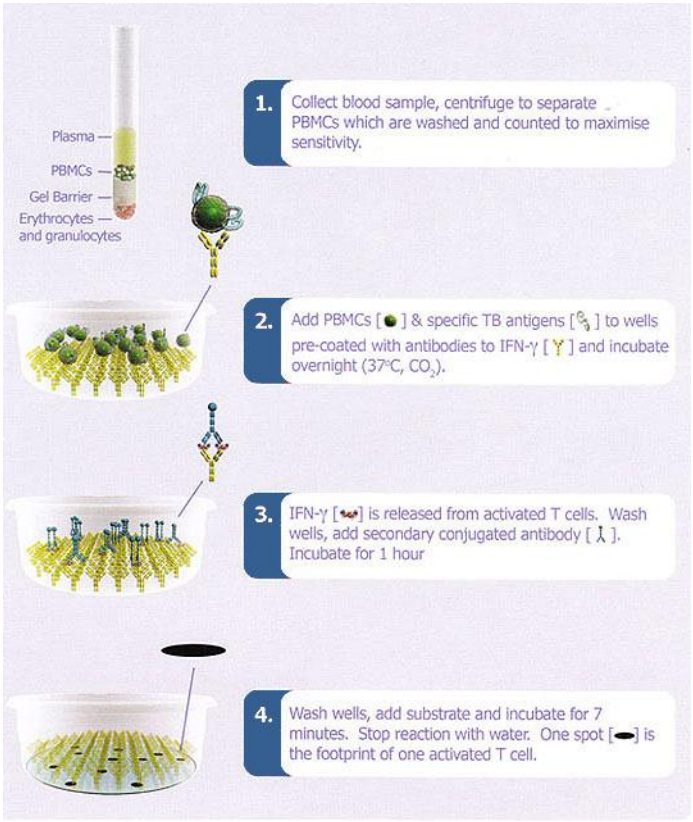


Figure 3. Principle of the T-Spot®.*TB* assay system. Source: www.oxfordimmunotec.com.

Table 2. Interpretation criteria for the T-Spot®.*TB* assay.

result	**spot count**				
	MTB antigens			*nil*	*PHA*
	ESAT-6		CFP-10		
positive	≥ 6 over nil	and/or	≥ 6 over nil	≤ 10	any
negative	≤ 5 over nil	and/or	≤ 5 over nil	≤ 10	≥ 20
indeterminate	≤ 6 over nil	and	≤ 6 over nil	≤ 10	< 20
	any		any	> 10	any

As this assay controls for overall numbers of PMBCs in an individual stimulatory reaction, the T-Spot.*TB* test is less susceptible to a loss of $CD4^+$ T-cells as caused e.g. by HIV infection and has thus been recommended as the preferred option for diagnosis of LTBI in HIV/MTB co-infected individuals (LEIDL et al., 2010).

The increase in specificity as compared to skin testing has raised some hope that IGRAs may have potential to be used in the diagnosis of active TB. Although individual studies have provided evidence that neither the TST nor IGRAs can discriminate between active and

latent infection (MACK et al., 2009), this has not been formally assessed in larger systematic reviews and meta-analyses. In addition, a systematic review of studies on the use of IGRAs from extrasanguinous fluids had not yet been performed.

1.6 Emerging drug resistance

Though the first antibiotic and thus the first effective treatment against TB was discovered as early as in 1944, treatment of TB has recently been confronted with severe setbacks. The past two decades were marked by a worldwide emergence of multi drug resistant (MDR) tuberculosis, then extensively resistant (XDR) TB, and, most recently, strains that are resistant to all anti-TB drugs (MIGLIORI et al., 2007; SHAH et al., 2007; GANDHI et al., 2010).

TB drug resistance can either be the result of primary infection with a resistant strain, or develop during treatment. Resistance of a primarily susceptible bacillus can be caused by a point mutation, or by a nucleotide deletion or insertion. Under normal conditions the mutant organisms are by far outnumbered by drug-susceptible bacilli. In the presence of an antimicrobial agent, however, susceptible organisms are reduced in number, which leads to a selective pressure in favor of the mutant organism. A TB patient who is treated with one single antibiotic drug will experience an initial response to treatment, but resistant bacteria can continue to replicate in the presence of the drug and can become predominant, resulting in a recurrence of the disease which is then fully resistant to the drug (NACHEGA, CHAISSON, 2003). Mutations for resistance to isoniazid, rifampicin, ethambutol or streptomycin are however very infrequent in MTB (estimated prevalence of mutation of 1 in 10^6 - 10^8 bacilli), and the probability of random mutations in an individual bacillus resulting in resistance to more than one drug consequently is extremely low. Standard treatment therefore consists of at least two, mostly three different antibiotics, so that any single pathogen that develops resistance to one of the drugs is at the same time confronted with at least one other antibiotic to which it should be susceptible. However, experience has shown that failure of local health-care systems in high burden settings can quickly lead to a systematic selection and spread of lethal multi or extensively drug-resistant strains (RUSSELL et al., 2010).

MDR-TB is defined as resistance to at least the two first-line drugs (isoniazid and rifampicin), while XDR-TB is caused by bacteria that in addition are resistant to any fluoroquinolone and at least one of three injectable second-line drugs (i.e. amikacin, kanamycin, or capreomycin).

It is estimated that there were about 0.5 million incident cases of MDR tuberculosis and 50,000 cases of XDR-TB in 2007. Among the MDR cases, about 0.3 million were new cases, i.e. showing primary drug resistance, and 0.2 million were patients previously treated for tuberculosis, i.e. with drug resistance acquired during previous treatment (WORLD HEALTH ORGANIZATION, 2011b). The latest WHO estimates suggest that globally and in the region of the Americas, levels of MDR-TB among new TB patients are relatively stable, and that numbers are falling in the Eastern Mediterranean, South-East Asia and Western Pacific regions while increasing in Africa and the European Region (WORLD HEALTH ORGANIZATION, 2011b).

Nevertheless, numbers were only reported as summary values for the WHO European Region and selected hot spot countries in that region. A detailed overview of the situation in the 27 member states of the European Union (EU27) was not available and should thus be compiled.

1.7 Co-infection with HIV

HIV, the pathogen which causes the Acquired Immune Deficiency Syndrome (AIDS), was first described in 1983 by Luc Montagnier and Françoise Barré-Sinoussi from the Institut Pasteur in Paris, and its devastating effects on the global TB balance became clear shortly thereafter. Today, it is well recognized that AIDS and TB form a lethal combination, each boosting the other's progress, and that the emergence of AIDS has led to a major upsurge in TB cases and TB mortality again in many countries (LAWN et al., 2011; WORLD HEALTH ORGANIZATION, 2011b).

HIV is a retrovirus that infects $CD4^+$ T-cells, dendritic cells, and macrophages. In addition to the profound functional and numeric depletion of $CD4^+$ T-cells, it is assumed that HIV targets and depletes MTB antigen-specific $CD4^+$ T lymphocytes earlier and at a greater frequency than $CD4^+$ T-cells specific for other antigens (ERNST, 2012). Consequently, HIV co-infection directly impacts the ability of the immune system to control MTB infection (LAWN, ZUMLA, 2011). Thus, the virus renders patients more susceptible to infection, and also increases the likelihood of 'latently' infected individuals to develop active disease through reactivation. Fifteen million people are estimated to be co-infected with MTB and HIV, with the risk to develop active TB increasing from an approximately 10% lifetime risk in immunocompetent

individuals to over 10% each year in a co-infected patient (VAN SOOLINGEN et al., 1997; WORLD HEALTH ORGANIZATION, 2009).

Co-infection with HIV has a profound impact on the spectrum of TB disease, with HIV-induced progressive immunodeficiency increasing the risk of extrapulmonary and disseminated disease (PHILIPS, ERNST, 2012). TB is the most common opportunistic disease and the leading cause of death for people infected with HIV, resulting in an increased morbidity and mortality of TB infection both globally and in Europe (TOOSSI, 2003).

Furthermore, TB diagnosis is more difficult in individuals co-infected with HIV. HIV-associated TB is more likely to be smear-negative than TB in individuals who are HIV-negative due to a reduced inflammatory response with lower rates of cavitation in the lung (GETAHUN et al., 2007). Despite lower bacillary burden in sputum, the total numbers of mycobacteria in patients with advanced HIV infection and TB may be very high (LAWN et al., 2011). Postmortem studies of HIV-infected patients consistently found TB to be the most common cause of death. In most cases, patients were affected by undiagnosed disseminated TB (RANA et al., 2000; ANSARI et al., 2002). These studies emphasize that HIV-associated disseminated TB may significantly contribute to HIV-associated mortality but frequently remains subclinical and undiagnosed. Thus, the WHO estimate of approximately 0.5 million deaths per year in patients with HIV-associated TB may even be an underestimate (LAWN et al., 2008).

Tests which rely on the measurement of MTB-specific immune reactions such as the TST or IGRAs naturally often fail in immunocompromised patients. In some HIV/MTB endemic countries, this leads a situation where less than 40% of all cases are detected, thus leaving the majority of HIV-associated TB cases undiagnosed (MCNERNEY et al., 2012). Hence, HIV co-infected individuals represent a large reservoir of undetected infectious agents, are much more prone to conversion, and, once active TB has developed, may further spread the bacillus again.

To further complicate the situation, not only the infection itself, but also a successful onset of anti HIV treatment can lead to TB-associated damage: When HIV and MTB co-infected individuals are treated with antiretroviral drugs against HIV, they are at risk of developing an immune reconstitution inflammatory syndrome (IRIS), where the recovery of the immune system leads a sudden increase in the inflammatory response, causing either non-specific

symptoms such as fever, or in some cases a worsening of damage to the infected tissue. In severe cases, this may even result in increased morbidity (MEINTJES et al., 2008).

For all these reasons, the HIV pandemic presents a significant challenge to global TB control (WORLD HEALTH ORGANIZATION, 2012c), and special measures need to be taken both for disease control and further research.

Despite the fact that AIDS and TB are considered poverty related diseases, this also applies to Europe, where TB is declining in Western and Central Europe, but still high and increasing in Eastern Europe. HIV/AIDS, which has been prevalent in the past in countries in Western Europe, is increasing dramatically in Eastern Europe, boosting the spread of TB, including MDR-TB and XDR-TB. In view of these developments, the WHO in 2003 announced that they expect a significant acceleration of morbidity and mortality attributed to HIV/MTB co-infection in Europe in the future (WORLD HEALTH ORGANIZATION, 2003).

Thus, an evidence-based process to identify priority research issues in this field, driven by Europe and realized in an international process including stakeholders from both disciplines and those areas in the world that are most affected by the dual infection, seemed necessary and timely.

1.8 Surveillance

Recording and reporting of data are fundamental components of patient care and disease control, and an important basis for research planning. Data collection and reporting are necessary to monitor trends in the TB epidemic at all levels (global, national, and subnational), and to document treatment progress. Furthermore, those data represent a sound basis to plan, implement and evaluate efforts for disease control and to allocate resources accordingly. Functioning surveillance allows an early identification of hotspots or new trends and developments that require particular action. When high-quality data are available, corrective actions can be taken where needed, and successes can be documented (WORLD HEALTH ORGANIZATION, 2012a). This applies both to health policy measures as well as to research and development (R&D) questions. In the context of TB research, it allows detailed description of the populations included in clinical studies and provides an important basis for decisions on research questions and allocation of funding.

The ECDC, founded in 2005, and WHO/Europe jointly coordinate TB surveillance in the WHO European Region (27 EU member states, 3 European Economic Area (EU/EEA) countries, and 24 additional states, see Figure 4). Both organizations have agreed to share the data submitted by the 54 Member States of the WHO European Region and all tasks related to surveillance and monitoring of TB. For this purpose, a joint TB information system has been developed using a web-based common reporting entry point. TB surveillance data from the EU27 and EEA countries are processed through The European Surveillance System (TESSy) platform hosted by ECDC (established in 2008). Data from all other countries are processed through WHO's Global Tuberculosis database. Submitted data are validated and exchanged between ECDC and WHO/Europe.

Figure 4. EU27 in the WHO European Region.

The 27 EU Member States (dark blue): Austria, Belgium, Bulgaria, Cyprus, Czech Republic, Denmark, Estonia, Finland, France, Germany, Greece, Hungary, Ireland, Italy, Latvia, Lithuania, Luxembourg, Malta, the Netherlands, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden and the United Kingdom. **EEA (European Economic Area) countries** Iceland, Liechtenstein and Norway and **the 24 remaining countries in the WHO European Region (non-EU/EEA)** (light blue): Albania, Andorra, Armenia, Azerbaijan, Belarus, Bosnia and Herzegovina, Croatia, Georgia, Israel, Kazakhstan, Kyrgyzstan, the former Yugoslav Republic of Macedonia, Moldova, Monaco, Montenegro, Russia, San Marino, Serbia, Switzerland, Tajikistan, Turkey, Turkmenistan, Ukraine and Uzbekistan.

However, data are only published for the WHO European Region as a whole, and selected high burden countries within that area, but a specific and comprehensive overview of the epidemiological situation of the European Union was not available and thus to be compiled in the framework of this thesis.

1.9 Political and financial support

Funding for TB research was very minor until TB was declared a global emergency in 1993. With increasing awareness, the first research groups were funded to translate the results of basic research into new applications. Today, international collaboration is a key element of all large funding programmes, as is increasingly also the cooperation between the academic and the industrial sector (KAUFMANN, PARIDA, 2007). The last two decades have been characterized by visible political support, an increasing effort to streamline activities, and a strong increase in private funding, leading also to the creation of public-private-partnerships (PPP), which help optimize the use of scarce resources.

On the **political level**, global support was expressed in the United Nations Millennium Declaration in 2000, and has subsequently been confirmed on a number of occasions at high-level summits such as the annual G8 meetings and the Berlin declaration on TB (WORLD HEALTH ORGANIZATION, 2007). The Stop TB Partnership has established dedicated working groups for new TB diagnostics, new TB drugs, new TB vaccines, and most recently a TB/HIV Working Group with an Infection Control Subgroup.

As a consequence, **new public funding programmes** with a clear focus on infectious diseases have been initiated, such as the European and Developing Countries Clinical Trials Partnership (EDCTP), a partnership between currently 16 European states and a large number of sub-Saharan African countries. The EDCTP is specifically dedicated to poverty related diseases (PRD) research with a clear focus on and strong participation of Sub-Saharan Africa and an attempt to build sustainable capacities in this endemic region.

In recent years, **private investors** such as the Bill & Melinda Gates Foundation (BMGF) or the Global Fund for AIDS, TB and Malaria have started to significantly contribute to TB and/or HIV/AIDS funding. While BMGF has quickly developed to be a key player in global TB research funding, the Global Fund has a strong focus on HIV/AIDS, with only 15% of the total funding going to TB, of which again the majority is dedicated to TB control programs (KAUFMANN, PARIDA, 2007).

In several successful cases, the public and private sector have joined forces and combined resources in newly created non-profit **public private partnerships** (PPP), such as the Foundation for Innovative New Diagnostics (FIND, 2003), which drives the development and

early implementation of innovative diagnostic tests for TB and other diseases, the TB Alliance (2000) which is dedicated to the discovery and development of new, faster-acting and affordable TB drugs, and Aeras (2003), a product development organization dedicated to the development of effective TB vaccines.

Despite these efforts, however, the total funding available for TB research and development still falls short of the US$ 1.8 billion per year that is called for in the Global Plan to Stop TB 2011–2015 (WORLD HEALTH ORGANIZATION, 2011a). With a clear shortage of funds, thorough monitoring of research-spending and informed evidence-based decision making become increasingly important.

In order to **monitor the spending of research funds**, the BMGF has commissioned the George Institute for International Health to conduct annual surveys of global investment into R&D of new pharmaceutical products to prevent, manage, or cure diseases of the developing world (MORAN et al., 2009a). This G-FINDER survey is intended to report accurate, comparable R&D investment figures across the spectrum of neglected diseases, including HIV/AIDS and TB funding, as a starting point for informed decision making of funders and politicians.

As a second large initiative, and also funded by the BMGF, the Treatment Action Group (TAG) has been providing an annual overview of the global TB funding expenditures since 2005. TAG is a New York based independent AIDS advocacy group fighting for better treatment, a vaccine, and a cure for AIDS. In the context of their TB/HIV project, TAG has been compiling the 'Treatment Action Group's report on funding trends for tuberculosis (TB) research and development (R&D)', monitoring global spending for TB R&D from the baseline year 2005 through currently 2010 (JIMÉNEZ-LEVI, 2012).

In that sense, **streamlining and coordination of efforts** have become central elements of funding strategies in Europe. To overcome fragmentation and optimize the efficiency of use of resources, the EC has created large Framework Programmes (FP), bundling all research activities to large multi-annual programmes. They are characterized by a strong emphasis on collaborative research between scientists in different countries and different sectors. Since their introduction in 1984, they have become the main tool to fund cooperative research in Europe (LANG et al., 2010). Within the current FP7, the cooperation scheme is unique in its internationality, and has been taken up very positively by the scientific community (HANSEN

et al., 2012). HIV/AIDS, malaria, and tuberculosis became a specific research focus already in FP6, which ran from 2002 – 2006 and allocated €450 million in PRD R&D. To continue these efforts within FP7, the EC organized a conference on PRD in Brussels on Nov 13-14 2008, entitled Challenges for the Future: research on HIV/AIDS, malaria and tuberculosis, with the aim to develop views on how to focus PRD research under the new FP7 (GRYSEELS et al., 2009).

In that context and in the same month, the EC has initiated funding for the 'European Network for global cooperation in the field of AIDS and TB' (EUCO-Net; www.euco-net.eu), a project that brought together experts from Europe and several regions with a particularly high burden of HIV/MTB co-infection, to develop an **evidence-based joint research agenda for AIDS and TB research** (SESTER et al., 2010). It is in the framework of this project that the majority of the work presented in this thesis has been performed.

1.10 Specific aims

TB surveillance data collected by WHO and ECDC are published annually, but data are shown only for the WHO European Region in summary, and selected individual countries. Particularly, no overview of the situation of HIV/MTB co-infection has been available. TB diagnostic practice is inconsistent, with a variety of diagnostic tests in use, but non-existing or differing national guidelines throughout Europe. To facilitate evidence-based, informed decision making both with regards to development of EU-specific guidelines and the elaboration of a joint research agenda, this thesis aimed to

- provide an **overview of the epidemiology** of TB (including MDR/XDR-TB) and HIV/TB co-infection in all 27 EU member states,
- compare **TB diagnostic standards** and the availability of tests in Europe and high burden areas,
- assess the **suitability of IGRAs for the diagnosis of active TB** as a basis for new EU-specific diagnostic guidelines,
- prepare and coordinate the development of recommendations for future HIV/TB research activities (in Europe and globally) and synthesize them in a **Roadmap**,
- align these suggestions with ongoing initiatives and trends regarding HIV/AIDS and TB funding within the EU research **funding portfolio**, and to
- summarize the most relevant facts about HIV/MTB co-infection in the form of a **short film** geared towards the media and key decision makers.

The main aims, outputs and addressees of this thesis are summarized in Figure 5.

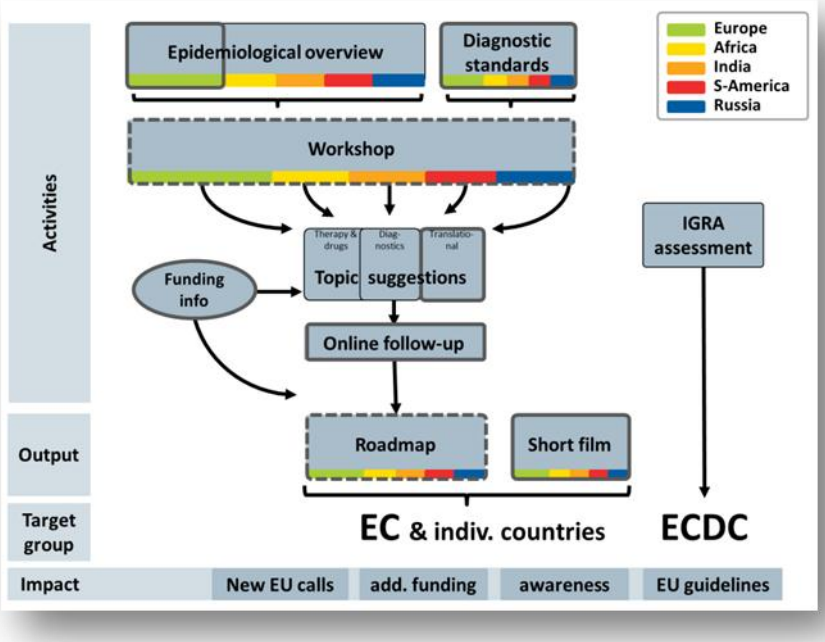


Figure 5. **Activities described in this thesis, including their interrelation, results and impact. Solid frame: activity mainly performed by or led by C Giehl. Dotted frame: main responsibility shared between C. Giehl, A. Meyerhans, and M. Sester.**

2 Materials and methods

2.1 Abbreviations

AFB	acid-fast bacilli
AIDS	acquired immunodeficiency syndrome
BAL	bronchoalveolar lavage
BCG	Bacille Calmette-Guérin
BMGF	Bill and Melinda Gates Foundation
CFP-10	culture filtrate antigen 10
CSF	cerebrospinal fluid
DST	drug susceptibility testing
EC	European Commission
ECDC	European Centre for Disease Prevention and Control
EEA	European Economic Area
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunospot (assay)
ESAT-6	early secretory antigenic target 6
EU	European Union
EU27	the 27 member states of the European Union
FP7	the Seventh Framework Programme of the European Union
G-FINDER	Global Funding of Innovation for Neglected Diseases
HIV	human immunodeficiency virus
ICD-10	International Classification of Diseases, 10th edition
IFNγ	Interferon gamma
IGRA	Interferon gamma release assay
LTBI	latent tuberculosis infection
M.	Mycobacterium
MDG	Millennium Development Goal
MDR-TB	multidrug-resistant tuberculosis
MTB	Mycobacterium tuberculosis
NGO	non-governmental organization
PBMC	peripheral blood mononuclear cell

PHA	phyto-hæmagglutinin
PPD	purified protein derivate
PPP	public private partnership
PRD	poverty-related diseases
QFT-GIT	QuantiFERON Tb-Gold In-Tube
R&D	research and technological development
TAG	Treatment Action Group
TB	tuberculosis
TBNET	Tuberculosis Network European Trials Group
UNAIDS	Joint United Nations Programme on HIV/AIDS
WHO	World Health Organization
XDR-TB	extensively drug-resistant TB

2.2 Epidemiological data

2.2.1 Data collection

A collection of TB epidemiological data and related information on HIV/MTB co-infection was compiled for 2008 as a reference year - the latest year for which the full range of data was already available at the time of collection - for all 27 EU member states, including both surveillance data (i.e. reported cases) and WHO estimates.

Reported case numbers for TB, MDR-TB, XDR-TB, and TB-related deaths in the European Union were extracted from the 'ECDC surveillance report: tuberculosis surveillance in Europe 2008' (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2010b), and complemented with case numbers provided by countries to WHO as available in the global WHO database (freely accessible at http://www.who.int/tb/country/data/download/en/index.html). Where data for 2008 were not available, the value of the closest possible year was collected, preferably for the next following year, and the deviation from the standard reference year is clearly indicated in the respective tables.

Estimated case numbers generated by WHO for the same reference year were extracted from the WHO report 2011 on global tuberculosis control (WORLD HEALTH ORGANIZATION, 2011b) and the global WHO database. Following WHO recommendations (WORLD HEALTH ORGANIZATION, 2012a), estimated case numbers for the reference year 2008 presented or used herein are taken from the latest WHO report only (which in many cases provides updates on estimates reported in previous reports), i.e. updated as compared to data previously published in the context of this thesis (GIEHL et al., 2011). The accuracy of estimates is said to have significantly improved due to a clear increase in availability of data, and improved calculation methods. Furthermore, for better readability, only best estimates are indicated here (without lower and upper bounds as calculated by WHO, defined as the 2.5th and 97.5th percentiles of outcome distributions produced in simulations). 2008 population data for the calculation of prevalence and incidence rates (see below) were obtained from the WHO global database, with totals available for all 27 EU countries.

2.2.2 Calculation of prevalence, incidence, and mortality

Prevalence (TB; MDR-TB; XDR-TB) was calculated as the total number of cases (all forms of TB; MDR-TB; XDR-TB) per 100,000 population. Incidence (TB; MDR-TB) was calculated as the

number of new and relapse cases (of all forms of TB; MDR-TB) per 100,000 population. Mortality was calculated as the total number of reported deaths caused by the disease in HIV-negative people per 100,000 population. TB deaths among HIV-positive people are to be classified as HIV deaths according to the latest revision of the international classification of diseases (ICD-10) and are thus not included in TB mortality calculations.

Prevalence and incidence were calculated both from reported cases and from estimates (resulting in 'estimated prevalence' and 'estimated incidence'), with the basis for calculations clearly indicated in presented tables or graphs.

2.3 Diagnostic standards and disease management

2.3.1 Data collection on diagnostic standards

To assess country standards with regards to use of diagnostic tests and resistance testing in Europe, and to compare those with the situation in several high burden regions, an electronic survey was performed among health care practitioners in Europe, India, Russia, South America, and Sub-Saharan Africa.

Data collection

Respondents were asked to indicate which test is used for standard diagnostic purposes in their state sector, both for contact tracing and for diagnosis of active TB in adults, and how commonly the respective test is used (Figure 6):

1 – very common

2 – common

3 – rare

4 – not at all / not available

In addition, a free text field allowed the reporting person to further specify the transmitted information, or report on difficulties in information retrieval, or inconsistencies of available data.

contact tracing			TB diagnostics						resistance testing	
TST	X-ray	IGRA	TST	X-ray	IGRA	microscopy	culture	PCR	phenotypic	genotypic

Figure 6. Table to be filled in by health care practitioners in the electronic survey.

The questionnaire was sent to healthcare practitioners in those European countries with the highest TB prevalence, highest number of reported TB cases, or highest MDR-TB prevalence (top five each) as well as in India, Russia, South America, and selected Sub-Saharan African countries.

2.3.2 Meta-analysis of data on diagnostic tests (TST and IGRAs)

To assess the capacity of commercially available IGRAs to diagnose active TB in humans in blood and in extrasanguinous fluids, a systematic review was conducted together with a working group of the Tuberculosis Network European Trialsgroup (TBNET), a non-profit, non-governmental peer-initiated scientific organization to collaboratively address research priorities in the area of tuberculosis in Europe (SESTER et al., 2011; GIEHL et al., 2012). The review was commissioned by the ECDC and performed according to the 'Preferred Reporting Items for Systematic Reviews and Meta-Analyses' (PRISMA) statement (MOHER et al., 2009) and the 'Quality Assessment of Diagnostic Accuracy Studies' (QUADAS) checklist (WHITING et al., 2003).

Study selection

To identify studies for potential inclusion, PubMed, EMBASE and the Cochrane-controlled central register of controlled trials were searched using the keywords 'tuberculosis', 'T-spot', 'Quantiferon', 'interferon-gamma release assay', 'IGRA', 'ESAT-6' and 'CFP-10'. Existing systematic reviews and meta-analyses were screened manually for additional references.

The results from this search were compiled into an initial database from which duplicate citations were eliminated (both 'real' duplicate listings of the same study and any study using the same patients as an already included study). Title and abstract of each citation were screened independently by two experts to select relevant studies. Where different judgments on the inclusion of a study were made, abstract and title were revisited jointly by the two experts to reach a consensus decision.

To be included, studies had to report on the assessment of commercially available IGRAs in individuals with a clinical suspicion of active tuberculosis, performed on blood or biological fluids other than blood (ascites, BAL-fluid, CSF, pericardial fluid, pleural effusion). Publications appearing in a language other than English or before January 2001 or after

November 2009 were excluded. In a final selection step, the full text articles of the remaining publications were screened to confirm their inclusion in the meta-analysis.

Studies were excluded if belonging to one of the following groups:

(1) case reports, editorials, or review articles
(2) laboratory studies
(3) animal studies
(4) studies using other assays than QFT-GIT or T-SPOT.*TB*
(5) studies which were not performed according to manufacturers' instructions
(6) studies where tuberculosis was not confirmed by MTB culture, histopathological findings and/or nucleic acid amplification tests (NAAT) in more than 50% of cases
(7) studies performed with cut-offs for positive test results that are not used in Europe
(8) studies where IGRA testing was performed after more than two weeks of tuberculosis treatment.

Criteria 5 and 7 were only applied to studies on IGRAs performed on blood.

Data extraction

From those studies that were finally included, data were extracted and merged into a joint excel spreadsheet. Key data collected comprised the study period, study design, country in which the study was conducted, gender distribution, age groups (adults vs. children), proportion of immunocompromised patients, BCG vaccination status, type of test performed, sensitivity and specificity for all tests compared, specimen on which the test was performed (for IGRAs), proportion of indeterminate results, proportion of AFB sputum positive patients, and proportion of culture confirmed cases. Where data could not be extracted directly from the publication, authors were contacted by email or telephone to retrieve the missing information.

Statistical analysis

Combined estimates of sensitivity, specificity and diagnostic odds ratio were computed. Indeterminate results were excluded before calculation of sensitivity and specificity. Forest plots were constructed to graphically assess both the variability of the estimates of the diagnostic parameters, and the weight of every sample size in the calculation of the pooled

estimates (weighted means). A random-effects meta-analysis was performed in order to account for the expected between-study variability for each study, along with a pooled estimate using the software for statistical analysis Stata 9.0 (StataCorp, College Station, TX, USA) and MetaDisc software, version 1.4 (ZAMORA et al., 2006). Inconsistency (statistical heterogeneity) among studies was assessed by the conventional chi-squared test for heterogeneity and by calculating the I^2 statistic in order to highlight the effect of true variability rather than sampling error on the overall variation in diagnostic estimates. Statistical analysis was performed by TBNET member Giovanni Sotgiu from the Institute of Hygiene and Preventive Medicine, University of Sassari, Sassari, Italy.

2.4 Definition of research priorities within the EUCO-Net project

Following a baseline assessment of epidemiological data on HIV/AIDS and TB, and of diagnostic standards both in Europe and four additional focus areas (Russia, India, South America and Sub-Saharan Africa), a collaborative process to identify joint research priorities was initiated within the FP7 project EUCO-Net, with the aim to elaborate a Roadmap on HIV/MTB co-infection, identifying global research needs and focus topics for future collaborative research (SESTER et al., 2010).

For that purpose, a panel of 60 experts was convened on July 23-24 2009 in Stellenbosch, South Africa, bringing together stakeholders from Europe and the four additional focus areas with complementary backgrounds, including scientists from academia, research institutes and the industrial sector, health care professionals, policymakers, activists, and people living with HIV/MTB.

2.4.1 Selection of experts for preparation of the EUCO-Net Roadmap

To ensure an appropriately balanced composition of the expert group, a systematic approach of expert selection was chosen, aiming at a total participant number of 60, with 20 experts from Europe, and 10 experts from Russia, India, South America, and Sub-Saharan Africa, respectively. Each region was to include the same number of HIV and TB experts. ‘Core group experts’, i.e. partners of the EUCO-Net project consortium, were to select their group peers; each expert was requested to suggest up to 6 ‘additional experts’, if possible from different sectors and with complementary backgrounds to achieve a mix of participants from industry, academia, patient associations, advocacy groups, including clinicians,

scientists, and policy makers. Where the number of suggested experts exceeded the available slots per region, the final selection was done by the coordinating team (M. Sester, A. Meyerhans, and C. Giehl), based on a pre-defined set of quality criteria.

The targeted number of participants was 60, of which 30 with a background in HIV/AIDS research, and 30 from the TB field. Europe was to be represented by 10 HIV/AIDS and 10 TB experts, while all other regions were invited to suggest 5 experts each (Figure 7).

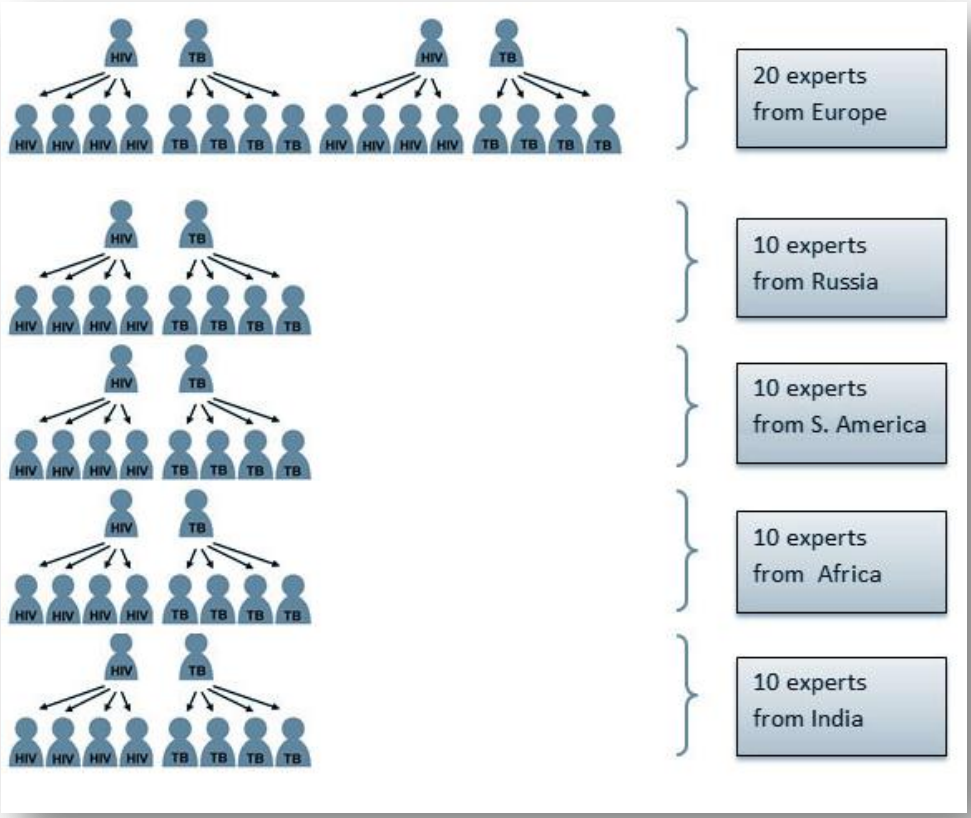


Figure 7. Approach for selection of workshop participants.

2.4.2 Assessment of baseline situation and definition of focus topics

To ensure that all experts were adequately informed of the existing knowledge base, and starting from the same point of knowledge, regional reports were presented by each of the five target regions, including important focus topics selected by the presenter.

Following this, experts were subdivided into ad-hoc committees by research subject area. The following three work groups were established: i) 'Therapy and drug interactions'; ii) 'Diagnostics'; and iii) 'Translation of research'.

Experts were allocated to the work groups prior to the beginning of the workshop, based on their publication history and current focus of activity. Where allocation to two or more groups was possible, additional factors such as the best possible balance between the different working groups or expressed personal preferences were taken into account.

Presented focus topics were discussed and complemented in each group, and each working group presented a maximum of five jointly elaborated topics at the end of the group session.

Following the workshop, the core group experts came together to a Roadmap Roundtable in order to refine the identified common research needs and to define final structure and content of the Roadmap document. A detailed work plan for the finalization of the Roadmap was agreed, including task allocation and exact time planning.

2.4.3 Online follow-up, consensus procedure, and writing of the Roadmap

Workshop participants received access to a password protected online platform which allowed them to further comment on the draft document that was produced as an outcome of the workshop and roundtable discussion. Topic suggestions as elaborated during the workshop were put up for further discussion and comments online from August 1 - September 30, 2009. Participants had the opportunity to edit the draft document by adding comments in 'track changes' mode, and upload edited draft versions under 'commented versions', or to initiate or participate in a discussion on a message board.

After closing of the online follow-up phase, the proposed ‘priority areas for joint AIDS/TB research and concerted actions’ were divided into four main sections (basic sciences, clinical research, diagnostics, training and networking). A writing committee consisting of work group and discussion leaders at the workshop, one NGO representative per disease, and the coordinating committee was nominated to lead the consensus and writing process of the document (P. Cuchi, C. Giehl, B. Kampmann, R. McNerney, A. Meyerhans, A. Kritski, C. Lange, G.B. Migliori, M. Sester, G. Walzl, C. Wingfield, in alphabetical order). Introductory paragraphs for the four sections were written by the writing group members. Final editing of the Roadmap was performed by M. Sester, A. Meyerhans, and C. Giehl.

2.5 Funding analysis

2.5.1 Global funding

Data extraction

One of the elements to be taken into account when elaborating priority topics for future research funding was the current allocation of total global funding to the fields of HIV/AIDS and TB research. For that purpose, data on funding allocation for HIV/AIDS and TB research were extracted from TAG reports (JIMÉNEZ-LEVI, 2012) and G-FINDER database (MORAN et al., 2009a) and merged into a joint excel spreadsheet. Key data collected comprised the total funding for HIV/AIDS, total funding for TB, and allocation of funding according to different fields of research (HIV/AIDS: basic research, diagnostics, microbicides, and vaccines; TB: basic research, diagnostics, drugs, vaccines, unspecified). Secondly, as the Roadmap was primarily directed to the EC, data on the allocation of EC-funding from FP6 & FP7 to different fields of research were extracted from the TAG report (data available for 2005 – 2010).

Calculations

Based on G-FINDER data, the relation of HIV/AIDS vs. TB funding was calculated. Secondly, G-FINDER & TAG TB funding data were compared with each other to check consistency or spot differences. Lastly, based on TAG data, the development of FP6/FP7 TB funding disbursement according to different fields of research since 2005 was calculated.

2.5.2 HIV/AIDS and TB in FP7

Project selection

To identify projects to be included in the overview, the project database of the infectious diseases unit of the EC's DG Research Directorate F Health (http://ec.europa.eu/research/health/infectious-diseases/index_en.html) was consulted in May 2012. The site lists all FP7 HEALTH projects dealing with HIV/AIDS, Malaria and Tuberculosis. Possible search functions include grouping according to disease. Thus, in a first steps, all projects listed as 'HIV/AIDS' projects and 'TB' projects were selected. In a second step, the full project database was searched using the keywords 'tuberculosis', 'TB', 'HIV', 'AIDS'.

Projects were selected based on the acronym and full project title resulting from the two searches. To confirm the inclusion, project abstract and information presented on the project websites were reviewed.

Data extraction

Data on ongoing and completed projects were extracted from detailed project factsheets as provided by the project database of the infectious diseases unit, or from project websites. Data collected include title and abstract of the project, funding scheme, total project funding ('maximum requested EC contribution'), project start date, study duration, country of coordinating institution, and countries of project partner institutions. All data were transferred into one excel spreadsheet to allow for both, joint and separate analysis and calculations.

Calculations

Allocation to HIV/AIDS or TB or co-infection was based on the information contained in the project factsheet and the public project website. Where title and/or summary indicated a clear main focus on TB, the project was categorized as 'TB (only)'; where title and summary of the project indicated a clear main focus on HIV/AIDS, the project was categorized as 'HIV/AIDS (only)'. For the calculation of 'HIV/AIDS-only funding' or 'TB-only funding', the EC contribution dedicated to co-infection projects was allocated 50/50 to each.

Total allocated funding was divided by the total running time per project, resulting in a project-specific average monthly funding that was distributed equally over the project lifetime. For the calculation of cumulative funding over time, the total funding sum for all ongoing projects was calculated for each month.

2.6 Short film production

As the second main EUCO-Net outcome to raise awareness for the issue of HIV-TB co-infection, in addition to the Roadmap, a short film was produced in collaboration with a professional film production company, and distributed to decision makers and the public at large. Before the actual filming, key issues such as the target audience, core information to be conveyed, and the means to convey the information were defined as follows:

Target audience: Funding agencies, policy makers, and the general public

Key information to be conveyed:

HIV/MTB co-infection is a problem

- in Europe and many high burden regions
- due to mutual and manifold interactions, the two diseases represent a particular challenge when affecting the same patient
- TB and HIV/AIDS are usually tackled individually (both in research as well as in the clinics), but joint efforts are needed
- Therefore: EUCO-Net has been initiated, as a global, interdisciplinary initiative
- EUCO-Net is coordinated by a team of 1 HIV and 1 TB expert at Saarland University
- Aims of the project: assessment of status quo, needs, and development of a "Roadmap" with recommendations for further research

Means to convey the information:

- A **comic strip** illustrating the immunological basics of HIV/MTB co-infection (reduce to the minimum and illustrate graphically)
- Kickoff **meeting scenes and interviews** with scientists (providing additional information, examples from different regions, explaining research needs)
- South Africa as an example setting, showing **people affected by the two diseases**.

For the **comic strip**, the immunological basics of HIV/MTB co-infection were outlined and prepared as drawings to develop the storyline. Examples are shown in Figure 8.

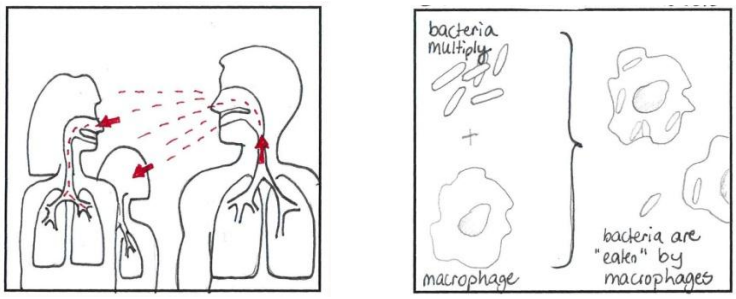


Figure 8. Drawings for the development of the comic strip storyline.

To guide the translation of the agreed elements into the final comic sequence, an animated presentation was prepared, linking the text to be included directly with the visual elements.

Meeting scenes and interviews with partners from the different target regions, all emphasizing the severity of the problem, were included to illustrate the international aspect

and global relevance of the co-infection issue. Two visits to homes of patients co-infected with HIV and MTB and a doctor's visit of a mother with her young son were included to show 'real' **people affected by the diseases**, adding an emotionalizing impression to the scientific contents of the film. Filming was prepared and coordinated by staff members of Stellenbosch University, including the collection of informed consent by the individuals to be featured in the film. To respect the stigma attached to TB and HIV, faces of the patients shown in the film were anonymized for those sequences that were not only meant for restricted scientific audiences. Locations and timeline of the filming are shown in Table 3.

Table 3. EUCO-Net filming: Locations, dates and scenes captured.

	N°	location	date	Scenes
filming	**1**	Brussels, Belgium	Nov 13-14, 2008	Interview scenes with selected experts representing each of the EUCO-Net target regions Brussels as headquarters of the European Commission and venue of the EUCO-Net kickoff-meeting
	2	Saarland, Germany	Dec 12, 2008	Location of the coordination team at Saarland University & Eurice GmbH; laboratory visit to the Fraunhofer Institute for Biomedical Research
	3	Cape Town, South Africa	Jan 19-23, 2009	Example of a high burden setting where patients, clinicians and basic researchers are very closely interlinked: Impressions from a community clinic and home visits to patients suffering from co-infection; portrait of a research nurse taking care of these patients, and taking samples to a laboratory at Stellenbosch University; interview with scientists at Stellenbosch University

3 Results

3.1 Epidemiology of TB and HIV/MTB co-infection in Europe

3.1.1 TB Epidemiology

22 out of the 27 countries (81.5%) in the European Union provided a full set of data of TB epidemiology (total TB cases at the time of data entry, new TB cases during year of reference, and TB-related deaths during the year of reference), while for Austria, Belgium, Luxembourg, Portugal and Slovakia, at least one of these values were unavailable (Table 4).

Table 4. EU27: Reported TB cases and deaths, calculated prevalence, incidence and mortality.

country	TB cases	calculated TB prevalence per 100,000	new TB cases	calculated TB incidence per 100,000	TB deaths (2007, unless indicated otherwise)	calculated TB mortality per 100,000	total population
Austria	-	-	-	-	49*	**5.87**	834,806
Belgium	1,006	9.5	779	7.3	-	-	10,602,167
Bulgaria	3,151	**41.5**	2,838	**37.4**	275	**3.62**	7,591,322
Cyprus	50	4.6	17	1.6	1	0.09	1,077,001
Czech Republic	868	8.4	807	7.8	70	0.67	10,377,359
Denmark	367	6.7	330	6.0	14	0.25	5,497,312
Estonia	444	**33.1**	354	**26.4**	43	3.20	1,342,145
Finland	350	6.6	331	6.2	56	1.05	5,316,334
France	**5,812**	9.4	3,355	5.4	667	1.07	62,098,413
Germany	4,543	5.5	3,623	4.4	139	0.17	82,475,271
Greece	669	5.9	538	4.8	96	0.85	11,291,807
Hungary	1,606	16.0	1,321	13.2	231*	2.30	10,021,886
Ireland	470	10.8	335	7.7	45	1.03	4,352,866
Italy	4,418	7.4	3,409	5.7	407**	0.68	59,891,479
Latvia	1,070	**47.1**	918	**40.4**	144	**6.34**	2,271,198
Lithuania	2,250	**67.0**	1,892	**56.3**	284*	**8.45**	3,359,799
Luxembourg	28	5.8	-	-	-	-	486,781
Malta	53	12.8	46	11.1	6	1.45	413,946
Netherlands	997	6.0	948	5.7	54	0.33	16,503,263
Poland	**8,081**	21.1	7,061	18.5	775	2.03	38,218,462
Portugal	2,995	28.2	2,703	25.4	-	-	10,634,612
Romania	**24,786**	**114.8**	18,774	**87.0**	1,639*	**7.59**	21,589,544
Slovakia	633	11.6	510	9.4	-	-	5,441,304
Slovenia	213	10.6	198	9.8	29	1.44	2,018,172
Spain	**8,214**	18.2	6,769	15.0	475	1.05	45,146,258

country	TB cases	calculated TB prevalence per 100,000	new TB cases	calculated TB incidence per 100,000	TB deaths (2007, unless indicated otherwise)	calculated TB mortality per 100,000	total population
Sweden	552	6.0	456	4.9	64**	0.69	9,236,872
UK	**8,655**	14.1	6,520	10.6	435*	0.71	61,270,318
total EU27	**82,281**	**16.8**	**64,832**	**13.2**	**5,998**	**1.23**	**489,360,697**

TB deaths among HIV-positive people are to be classified as HIV deaths according to the latest revision of the international classification of diseases (ICD-10) and are thus not included in TB mortality calculations.
Numbers referred to in the text are marked in red.
*: reference year 2008
**: reference year 2006

The top five countries within the EU27 with the highest number of TB cases, as well as with the highest calculated prevalence, incidence and mortality are summarized in Table 5. 30% of all **reported TB cases** in the EU27 are located in Romania, followed by UK and Spain with more than 10% each. Taking into account the different population numbers, the top five countries in order of decreasing **TB prevalence** according to reported cases (all types of TB) are Romania (114.8), Lithuania (67.0), Latvia (47.1), Bulgaria (41.5), and Estonia (33.1 per 100,000 population). The same five countries also show the highest **TB incidence** rates, decreasing in the same order as for prevalence.

When it comes to **TB mortality**, Romania is surpassed in mortality by Lithuania. Notably, Austria enters the stage as the country with the fourth highest TB mortality in Europe. Even though TB is a notifiable disease in Austria and needs to be reported by physicians to the district public health authorities, the country has not passed on the information on TB cases to WHO, and the latest available case numbers date back to 2006, thus were not included in this compilation.

Table 5. EU27: Top 5 countries for reported TB cases, calculated prevalence, incidence and mortality.

	TB cases	calculated TB prevalence per 100,000 population	calculated TB incidence per 100,000 population	calculated TB mortality per 100,000 population
rank 1	Romania	Romania	Romania	Lithuania
rank 2	UK	Lithuania	Lithuania	Romania
rank 3	Spain	Latvia	Latvia	Latvia
rank 4	Poland	Estonia	Estonia	Austria
rank 5	France	Bulgaria	Bulgaria	Bulgaria

With regards to **geographical distribution**, the burden of TB infection and disease is distributed unevenly within the EU27, with the highest rates of prevalence and incidence in Eastern European countries, Portugal and Spain (Figure 9 and Figure 10).

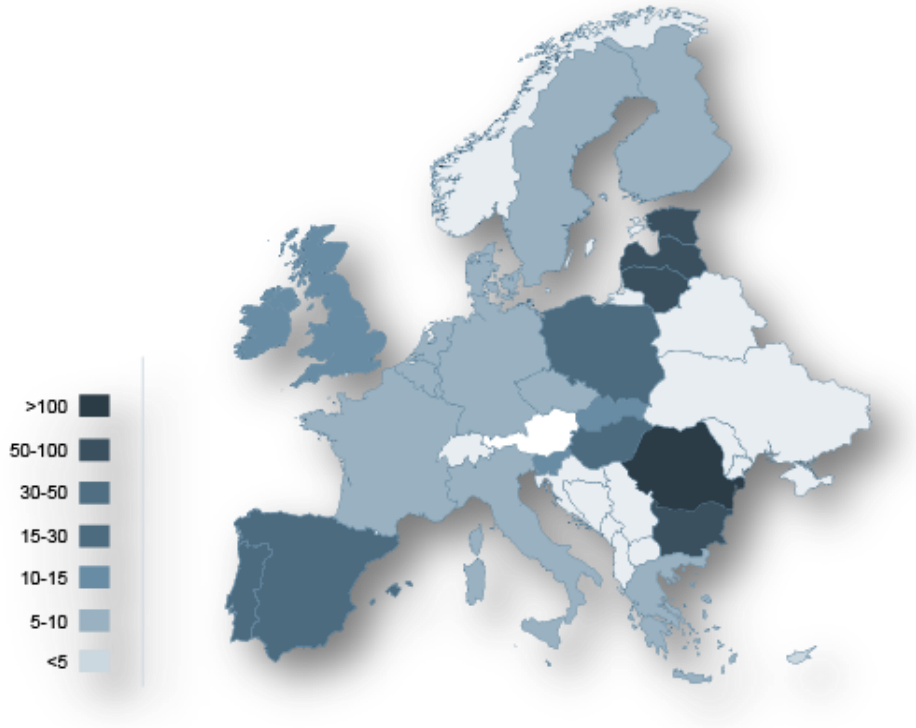


Figure 9. EU27: TB prevalence per 100,000 population in 2008.

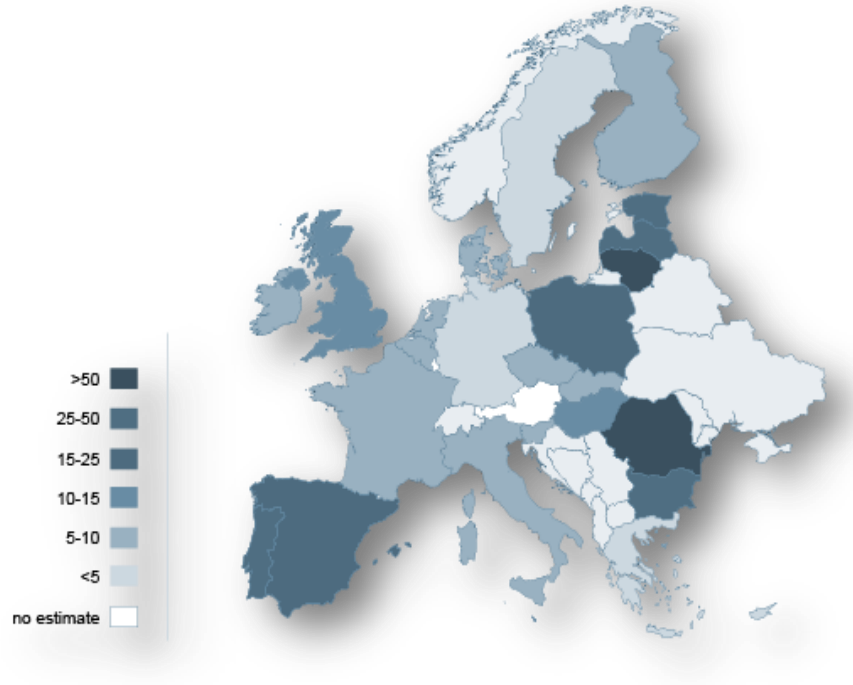


Figure 10. EU27: TB incidence per 100,000 population in 2008.

Drug-resistant tuberculosis in Europe

Data on drug-resistant TB in the EU are less complete; only 11 out of the 27 member states (40.7%) provided information on the full set of information (number of total MDR-TB cases, number of new MDR-TB cases, and total number of XDR-TB cases) in 2008. Italy reported no data on resistant TB at all, though cases are described in the literature (e.g.MIGLIORI et al., 2007). Information on new MDR-TB cases is further missing from Luxembourg, and data on XDR-TB were reported only by Belgium, Bulgaria, Cyprus, Czech Republic, Estonia, Latvia, Romania, Slovakia, Spain, Sweden, and the UK.

Table 6. EU27: Reported MDR & XDR-TB cases, calculated prevalence and incidence in 2008 (unless indicated otherwise).

country	MDR cases	calculated MDR prevalence per 100,000 population	new MDR cases	calculated MDR incidence per 100,000 population	XDR cases	calculated XDR prevalence per 100,000 population	total population
Austria	22*	**2.64**	5*	0.60	-	-	834,806
Belgium	22	0.21	15	0.14	2	0.02	10,602,167
Bulgaria	32	0.42	0	0.00	0	0.00	7,591,322
Cyprus	1	0.09	0	0.00	0	0.00	1,077,001
Czech Republic	11	0.11	10	0.10	1	0.01	10,377,359
Denmark	2	0.04	0	0.00	-	-	5,497,312
Estonia	**74**	**5.51**	42	3.13	9	0.67	1,342,145
Finland	1	0.02	1	0.02	-	-	5,316,334
France	27	0.04	16	0.03	-	-	62,098,413
Germany	45	0.05	16	0.02	-	-	82,475,271
Greece	14*	0.12	9*	0.08	-	-	11,291,807
Hungary	16	0.16	8	0.08	-	-	10,021,886
Ireland	3	0.07	2	0.05	-	-	4,352,866
Italy	-	-	-	-	-	-	59,891,479
Latvia	**129**	**5.68**	83	3.65	19	0.84	2,271,198
Lithuania	**276**	**8.21**	113	3.36	-	-	3,359,799
Luxembourg	0*	0.00	-	-	-	-	486,781
Malta	1	0.24	0	0.00	-	-	413,946
Netherlands	13	0.08	11	0.07	-	-	16,503,263
Poland	52	0.14	18	0.05	-	-	38,218,462
Portugal	28	0.26	19	0.18	-	-	10,634,612
Romania	**816**	**3.78**	130	0.60	54	0.25	21,589,544
Slovakia	4	0.07	1	0.02	0	0.00	5,441,304
Slovenia	2	0.10	1	0.05	-	-	2,018,172
Spain	**76**	0.17	31	0.07	3	0.01	45,146,258

country	MDR cases	calculated MDR prevalence per 100,000 population	new MDR cases	calculated MDR incidence per 100,000 population	XDR cases	calculated XDR prevalence per 100,000 population	total population
Sweden	12	0.13	7	0.08	1	0.01	9,236,872
UK	53	0.09	38	0.06	1	0.00	61,270,318
total EU27	**1,732**	**0.35**	**576**	**0.12**	**90**	**0.02**	**489,360,697**

*: reference year 2009
Numbers referred to in the text are marked in red.

Table 6 shows that Romania has the highest number of reported MDR-TB cases (816), followed by Lithuania (276), Latvia (129), Spain (76) and Estonia (74). Based on these reported case numbers, the highest MDR-TB incidence, however, has been reported for Lithuania (8.21 per 100,000 population), Latvia (5.68) and Estonia (5.51). With a prevalence of 2.64 per 100,000 population, Austria again ranks among the top five countries in the EU. The geographic distribution of MDR-TB across Europe is shown in Figure 11.

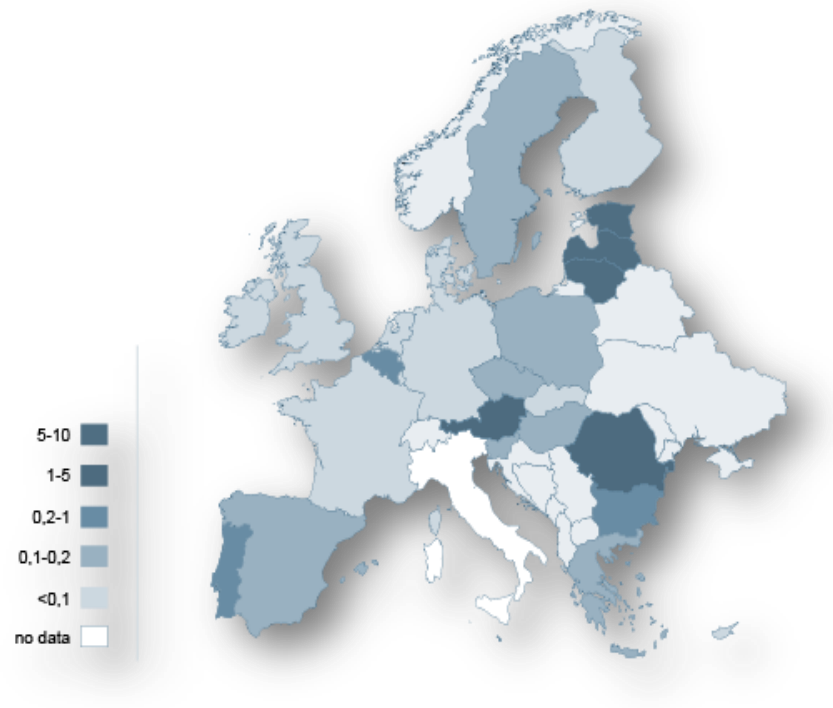


Figure 11. EU27: MDR-TB prevalence per 100,000 population in 2008.

3.1.2 Epidemiology of HIV/MTB co-infection

3.1.2.1 TB/HIV surveillance data

Surveillance data on HIV infected individuals with active TB ('TB/HIV') are very limited. Only 14 out of the 27 EU member states (51.9%) have reported case numbers at all, and these countries are scattered across Europe, with no identifiable distribution pattern (Figure 12).

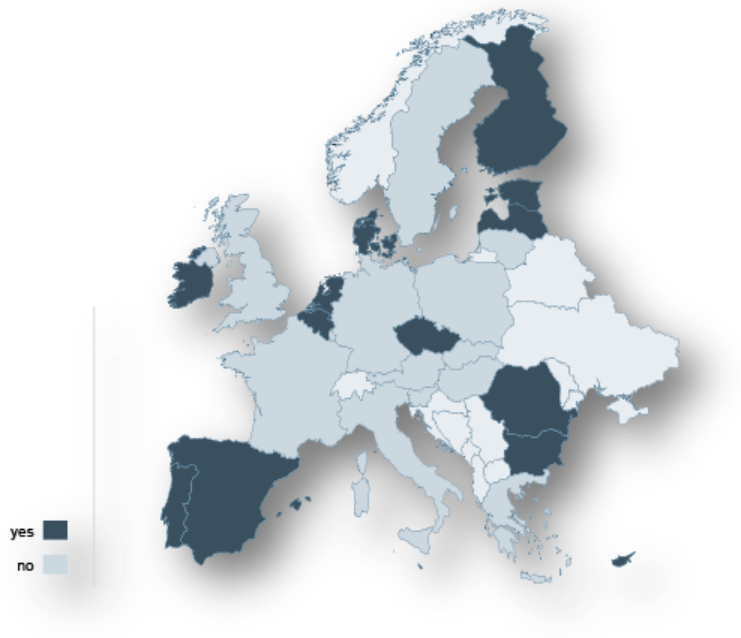


Figure 12. Availability of co-infection data

Of those countries for which information was available, Spain, Portugal and Romania had the highest absolute numbers of TB/HIV co-infected patients, with more than one third of all cases of co-infection reported in Europe declared in Spain (507 of 1,402; 36.2%; Table 7).

The percentage of TB patients for whom the HIV status was known for the reference year 2008 ranged from 0.4% (Poland) to 100% (Slovakia), with a total of 20.1% of all TB patients in the whole EU27.

Table 7. Reported TB/HIV co-infection cases, calculated prevalence, and coverage of knowledge of HIV status among TB patients in 2008.

country	total reported TB/HIV patients	TB/HIV prevalence per 100,000	total TB patients with known HIV status	total TB patients	% of TB patients with known HIV status
Austria	-	-	-	-	-
Belgium	56	0.53	913	1,006	**90.8**

country	total reported TB/HIV patients	TB/HIV prevalence per 100,000	total TB patients with known HIV status	total TB patients	% of TB patients with known HIV status
Bulgaria	0	0.00	520	3,151	16.5
Cyprus	2	0.19	36	50	72.0
Czech Republic	7	0.07	174	868	20.0
Denmark	13	0.24	158	367	43.1
Estonia	44	3.28	400	444	**90.1**
Finland	4	0.08	-	350	-
France	-	-	-	5,812	-
Germany	-	-	-	4,543	-
Greece	-	-	-	669	-
Hungary	-	-	-	1,606	-
Ireland	15	0.34	54	470	11.5
Italy	-	-	-	4,418	-
Latvia	72	3.17	910	1,070	85.0
Lithuania	-	-	-	2,250	-
Luxembourg	-	-	-	28	-
Malta	5	1.21	45	53	84.9
Netherlands	37	0.22	261	997	26.2
Poland	-	-	35	8,081	**0.4**
Portugal	438	4.12	**2,350**	2,995	78.5
Romania	202	0.94	**6,149**	24,786	24.8
Slovakia	-	-	633	633	**100.0**
Slovenia	-	-	-	213	-
Spain	**507**	1.12	3,937	8,214	47.9
Sweden	-	-	-	552	-
UK	-	-	-	8,655	-
total EU27	**1,402**	**0.29**	**16,575**	**82,281**	**20.1**

Numbers referred to in the text are marked in red.

3.1.2.2 TB/HIV estimates

While only 1,402 cases are reported from all 27 EU member states together, the WHO is estimating that 3,702 patients that are suffering from active TB are also co-infected with HIV in the European Union (Table 8). Spain, the country with the highest reported number of TB cases co-infected with HIV, also ranks number one in the estimate (a total estimated number of 990 patients, i.e. almost double the 507 reported case number). On average, the estimated values deviate by nearly 40% from the reported numbers, ranging from an under-estimation in the Czech Republic (where the best estimate of total co-infected patients is

lower than the reported number of cases) to an estimate that exceeds the reported number of cases by more than 200%, as shown for Romania (202 reported vs. 630 estimated cases).

Table 8. TB/HIV co-infection cases, surveillance data vs. estimates, and estimated mortality.

country	total reported TB/HIV patients	estimated total TB/HIV cases	calculated prevalence based on estimated TB/HIV cases	difference between reported cases and estimate	deviation between reported number and estimate [%]	estimated TB/HIV deaths	calculated TB/HIV+ mortality based on estimates per 100,000 population
Austria	-	35	**4,19**	-	-	3	0.36
Belgium	56	63	0,59	7	12.5%	5	0.05
Bulgaria	0	3.3	0,04	3.3	-	9	0.12
Cyprus	2	-	-	-	-	-	-
Czech Republic	7	5.5	0,05	**-1.5**	-21.4%	1	0.01
Denmark	13	18	0,33	5	38.5%	2	0.04
Estonia	44	49	**3,65**	5	11.4%	18	1.34
Finland	4	5.9	0,11	1.9	47.5%	1	0.02
France	-	380	0,61	-	-	57	0.09
Germany	-	99	0,12	-	-	11	0.01
Greece	-	14	0,12	-	-	12	0.11
Hungary	-	15	0,15	-	-	4	0.04
Ireland	15	17	0,39	2	13.3%	2	0.05
Italy	-	210	0,35	-	-	43	0.07
Latvia	72	82	**3,61**	10	13.9%	12	0.53
Lithuania	-	10	0,30	-	-	9	0.27
Luxembourg	-	2	0,41	-	-	1	0.21
Malta	5	5.1	1,23	0.1	2.0%	1	0.24
Netherlands	37	45	0,27	8	21.6%	3	0.02
Poland	-	170	0,44	-	-	34	0.09
Portugal	438	560	**5,27**	122	27.9%	81	0.76
Romania	202	630	**2,92**	428	**211.9%**	129	0.60
Slovakia	-	1	0,02	-	-	-	-
Slovenia	-	-	-	-	-	-	-
Spain	**507**	**990**	2,19	483	95.3%	122	0.27
Sweden	-	13	0,14	-		1	0.01
UK	-	280	0,46	-		30	0.05
total EU27	**1,402**	**3,702.8**	**0,76**		**Av: 39.5%**	**591**	**0.12**

Numbers referred to in the text are marked in red.

The 35 cases estimated for Austria and the resulting prevalence of 4.19 per 100,000 population make this country number two among all European countries, after Portugal, which with its 438 reported and 560 estimated patients co-infected with HIV ranks number

one (prevalence of 5.27 per 100,000 population). They are followed by Estonia (3.65), Latvia (3.61), and Romania (2.92 per 100,000) (Table 9).

Table 9. **The five countries within the EU27 with the highest prevalence and mortality, based on estimated cases and deaths.**

	Estimated TB/HIV cases	Estimated TB/HIV prevalence per 100,000 population	estimated TB mortality per 100,000 population
rank 1	Spain	Portugal	Estonia
rank 2	Romania	Austria	Portugal
rank 3	Portugal	Estonia	Romania
rank 4	France	Latvia	Latvia
rank 5	UK	Romania	Austria

The geographic distribution of TB/HIV co-infection estimates is shown in Figure 13.

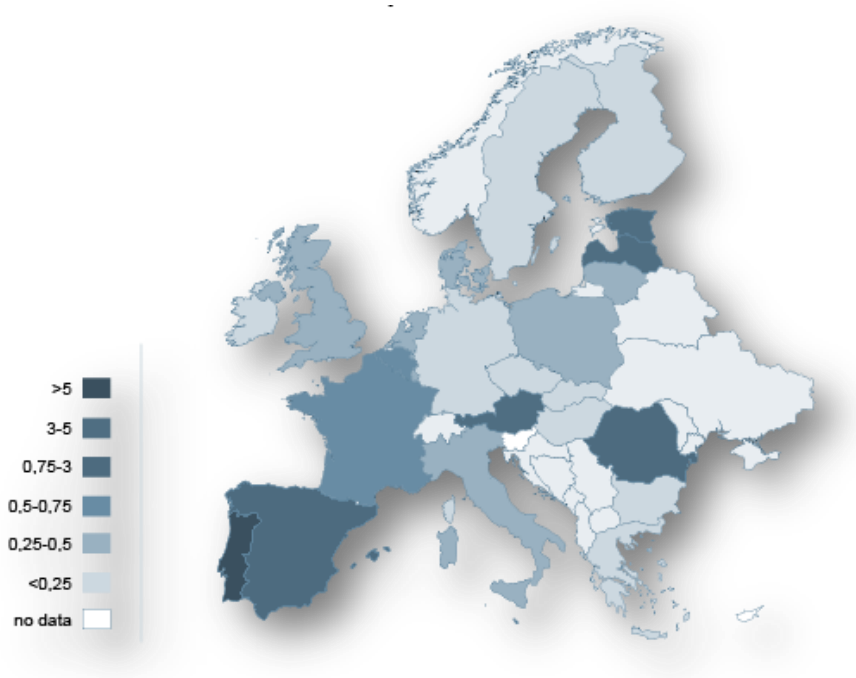


Figure 13. **Prevalence of TB/HIV co-infection, based on estimated case numbers.**

3.2 Variability of TB diagnostic standards and availability of tests

Countries included

While India and Russia are single countries, and South America comprises a number of countries that seemed still feasible to target, the high number of European (n=27) and Sub-Saharan African (n=48) countries required the selection of a sub-group of countries to be

included in data collection. For Europe, those countries were included that belonged to the top five countries in Europe for at least one of the following items: total number of reported TB cases, highest TB prevalence, or highest MDR/XDR prevalence. The selection of African countries to be included was based on availability of responsive contacts through existing collaborations and can be regarded as a random sample.

In total, data were requested for the following 30 countries:

- **Europe** (10 countries)**:**
  Austria (top 5 MDR/XDR prevalence), Bulgaria (top 5 TB prevalence and top 5 MDR/XDR prevalence), Estonia (top 5 TB prevalence and top 5 MDR/XDR prevalence), France (top 5 TB cases), Latvia (top 5 TB prevalence and top 5 MDR/XDR prevalence), Lithuania (top 5 TB prevalence and top 5 MDR/XDR prevalence), Poland (top 5 TB cases), Romania (top 5 TB cases and top 5 TB prevalence), Spain (top 5 TB cases), UK (top 5 TB cases).
- **India**
- **Russia**
- **South America** (12 countries)**:**
  Argentina, Bolivia, Brazil, Chile, Colombia, Ecuador, Guyana, Paraguay, Peru, Suriname, Uruguay, and Venezuela.
- **Sub-Saharan Africa** (6 countries)**:**
  Ethiopia, Malawi, Namibia, South Africa, The Gambia, Uganda.

Of those, 27 (90%) reported data back that could be included in the overview. No data could be retrieved for Guyana, Suriname, and India. In Guyana and Suriname, the survey was not completed. In India, the survey respondent stated that the RNTCP (Revised National TB Control Program) caters to ~40-50% of the country's TB. The rest is taken care of by the completely unregulated private sector, with no record of their numbers. Only very recently, on May 7th 2012, the government of India has declared TB a notifiable disease and officially banned TB serology, but the gap between health policy and its implementation is large. TB is still not notified by the private sector and serology is still being performed. The results retrieved for the remaining 27 countries are summarized in Table 10.

Table 10. **Frequency and type of test methods applied for contact tracing and TB diagnosis, and for resistance testing in Europe (10 countries), Africa (6 countries), Russia, and South America (10 countries).**

Europe:

Europe	contact tracing			TB diagnostics						resistance testing	
	TST	X-ray	IGRA	TST	X-ray	IGRA	microscopy	culture	PCR	phenotypic	genotypic
very common	90,0%	80,0%	10,0%	60,0%	100,0%	20,0%	100,0%	100,0%	20,0%	80,0%	20,0%
common	10,0%	20,0%	50,0%	10,0%	0,0%	20,0%	0,0%	0,0%	40,0%	20,0%	20,0%
rare	0,0%	0,0%	30,0%	30,0%	0,0%	30,0%	0,0%	0,0%	40,0%	0,0%	50,0%
not at all	0,0%	0,0%	10,0%	0,0%	0,0%	30,0%	0,0%	0,0%	0,0%	0,0%	10,0%

Africa:

Africa	contact tracing			TB diagnostics						resistance testing	
	TST	X-ray	IGRA	TST	X-ray	IGRA	microscopy	culture	PCR	phenotypic	genotypic
very common	16,7%	33,3%	0,0%	16,7%	66,7%	0,0%	100,0%	33,3%	16,7%	50,0%	16,7%
common	0,0%	0,0%	0,0%	0,0%	33,3%	0,0%	0,0%	16,7%	0,0%	0,0%	33,3%
rare	16,7%	0,0%	0,0%	66,7%	0,0%	0,0%	0,0%	33,3%	16,7%	16,7%	16,7%
not at all	66,7%	66,7%	100,0%	16,7%	0,0%	100,0%	0,0%	16,7%	66,7%	33,3%	33,3%

Russia:

Russia	contact tracing			TB diagnostics						resistance testing	
	TST	X-ray	IGRA	TST	X-ray	IGRA	microscopy	culture	PCR	phenotypic	genotypic
very common	100,0%	100,0%	0,0%	0,0%	100,0%	0,0%	100,0%	100,0%	0,0%	100,0%	0,0%
common	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	100,0%	0,0%	100,0%
rare	0,0%	0,0%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%	0,0%
not at all	0,0%	0,0%	100,0%	0,0%	0,0%	100,0%	0,0%	0,0%	0,0%	0,0%	0,0%

Latin America:

Latin America	contact tracing			TB diagnostics						resistance testing	
	TST	X-ray	IGRA	TST	X-ray	IGRA	microscopy	culture	PCR	phenotypic	genotypic
very common	80,0%	20,0%	0,0%	20,0%	90,0%	0,0%	100,0%	20,0%	0,0%	50,0%	0,0%
common	10,0%	70,0%	0,0%	60,0%	10,0%	0,0%	0,0%	50,0%	0,0%	30,0%	0,0%
rare	10,0%	0,0%	0,0%	20,0%	0,0%	0,0%	0,0%	30,0%	40,0%	20,0%	20,0%
not at all	0,0%	10,0%	100,0%	0,0%	0,0%	100,0%	0,0%	0,0%	60,0%	0,0%	80,0%

The information provided by respondents from all participating countries shows that overall, **all diagnostic tests assessed are most frequently used in Europe**, as compared to the other regions. While X-ray, microscopy and culture are routinely used for TB diagnostics by all European countries included in the survey, frequencies of use for the other tests vary.

The **use of IGRAs** shows the highest variability among all diagnostic methods used in Europe and ranges from very common (11.1% in contact tracing; 22.2% in TB diagnosis) to not at all/not available (11.1% in contact tracing; 33.3% in TB diagnosis). However, Europe is the only region that reported the use of IGRAs at all. In all of the non-European countries, IGRAs are 'not at all' used or 'not available' for both TB diagnosis and contact tracing.

Across regions, Sub-Saharan Africa is the area where **contact tracing** is least commonly performed with the test methods covered by the survey (no TST 66.7%; no X-ray 66.7%; no IGRA 100%). All other regions report TST and X-ray as commonly used methods for contact tracing (complemented by the frequent use of IGRAs for that purpose in Europe).

TB diagnosis in all countries assessed heavily relies on X-ray ('very common': 100% in Europe and Russia, 90% in Latin America, and 66.7% in Africa) and microscopy (100% 'very common' throughout).

Phenotypic **drug resistance testing** is more common than genotyping for the same purpose, and most common in Russia (100% 'very common') and Europe (7.8% 'very common', 22.2% 'common') while least frequently performed in Africa, where only 50% of the countries report phenotypic resistance testing as 'very common', but the other 50% perform either no resistance texting at all (33.3%) or only 'very rarely' (16.7%).

3.3 Limited suitability of IGRAs for the diagnosis of active tuberculosis

To assess the suitability of IGRAs for the diagnosis of active TB, a total of 844 studies were screened for analysis of patients with active TB using TST and/or IGRAs from blood and/or extrasanguinous fluid, of which 27 met the inclusion criteria (Figure 14, Annex I).

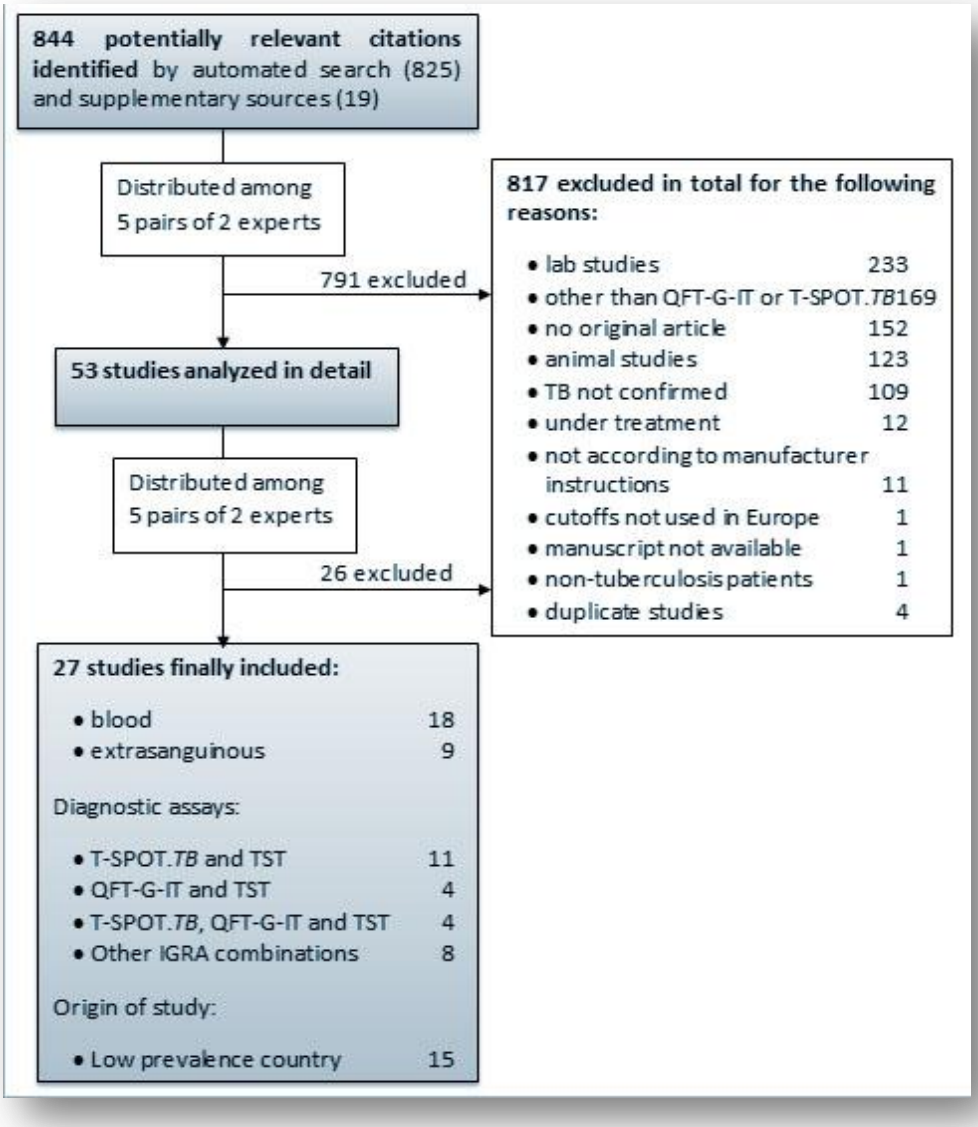


Figure 14. Flow chart of IGRA study inclusion and exclusion. Flow diagram for study selection.

Of these, IGRAs were performed on blood only in 18 studies (66.7%), while 9 (33.3%) included data on IGRAs performed on extrasanguinous fluids (5 BAL-fluids, 3 pleural fluids, 1 ascites). The most common combination of diagnostic assays was T-SPOT.*TB* and TST (11/27, 40.7%). 4 studies (14.8%) reported the use of QFT-GIT and TST, and the combination of all three assays, T-SPOT.TB and QFT-GIT and TST was used in another 4 studies (14.8%). 8 of the 27 studies (29.6%) reported the use of other IGRA combinations (Table 11).

Table 11. Diagnostic assays used in the studies.

diagnostic assays; number (%)			
	all individuals	adults	children
T-SPOT.TB and TST	11/27 (40.8)	10/23 (43.5)	1/4 (25.0)
QFT-GIT and TST	4/27 (14.8)	3/23 (13.0)	1/4 (25.0)
T-SPOT.TB, QFT-GIT and TST	4/27 (14.8)	2/23 (8.7)	2/4 (50.0)
Others (IGRAs only)	8/27 (29.6)	8/23 (34.8)	0/4 (0.0)

Characteristics of included patient population

The 27 studies included in the analysis were conducted in 35 different countries. South Africa was the most frequently represented country (6/35, 17.1%), followed by Italy and Germany (5/35, 14.3%, each), and Korea (4/35, 11.4%). 15 studies (56%) originated from a low-prevalence country, while 12 (44%) were performed in a high-prevalence country.

In total, 3,821 subjects were included in all 27 studies, of which 2,262 were male and 1,559 were female. The majority of the studies (23/27, 85.2%) included adult subjects only, while 4/27 (14.8%) reported on tests performed on children. As it was clearly possible to differentiate between adult and paediatric studies, age-related sub-analyses were performed. Other characteristics such as immunosuppression, BCG vaccination, or on country of origin, were already aggregated so that it was not possible to differentiate how they affect the diagnostic accuracy of IGRAs.

- **Studies in adults**

Males were more represented than females (1,818 vs. 1,167). Almost half of the included subjects were BCG vaccinated (mean (SD) proportion of BCG immunized patients per study: 42.1% (19.9%)). Patients with impaired immunity were enrolled in 15/18 (83%) studies; among these, HIV positive individuals were included in 13 (86.6%) studies. The majority of the studies enrolling adults described the performances of both TST and T-SPOT.*TB* (10/23, 43.5%). Tuberculin PPD RT-23 SSI was used by 12/15 (80%).

- **Studies in children**

The male to female ratio was 444/392. Most subjects were vaccinated with BCG (mean proportion of BCG immunized patients per study: 74.2% (21.4%)). Only one of the four

studies (25%) included immunocompromised children. Fifty percent (2/4) of the studies evaluated the performances of T-SPOT.*TB*, QFT-GIT and TST at the same time. Tuberculin PPD RT-23 SSI was used by 3 of the 4 studies (75%).

Characteristics of the TST described in the included studies

The median (IQR) number of individuals who underwent TST was 98 (131). The reaction was read at 48-72 hours in almost half of the cases (44.4%). Pooled sensitivity of the TST was 65% (95% CI: 61%-68%; I^2=89%), while pooled specificity was 75% (95% CI: 72%-78%; I^2=89%). Pooled diagnostic odds ratio was 5.72 (95% CI: 3.78–8.65; I^2=46.1%) (Table 12).

Pooled sensitivity computed after the inclusion of the studies not showing specificity (n=4) was similar to the above mentioned figure (66%; 95% CI: 63%-69%; I^2=93.2%), while pooled specificity computed after the inclusion of the studies not showing sensitivity (n=2) was lower (66%; 95% CI: 63%-68%; I^2=94.3%).

Characteristics of IGRAs performed on blood samples

The median (IQR) number of individuals who underwent QFT-GIT was 94.5 (189). The total number of TB patients and controls with determinate results were 1,035 and 569, respectively. The pooled sensitivity and specificity were 80% (95% CI: 75%-84%; I^2=45.3%) and 79% (95% CI: 75%-82%; I^2=81.1%), respectively. Pooled diagnostic odds ratio was 11.5 (95% CI: 5.1–25.7; I^2=67.8%) (Table 12). The addition of the studies not showing specificity (n=5) did not dramatically change the pooled sensitivity (77%; 95% CI: 75%-80%; I^2=64.5%).

The median (IQR) proportion of QFT-GIT indeterminate results was 7% (12.6%). This was two times lower than in the samples derived from adults only (14.9% (13.6%)). The median (IQR) proportion of indeterminate results among children was 6.3% (3.6%). The number of children with determinate results was 491. Mean (SD) sensitivity in children was 79.9% (20.9%). Specificity was reported in only one paediatric study (85.8%).

The median (IQR) number of individuals who underwent T-SPOT.*TB* was 90 (138). The total number of TB patients and controls with determinate results were 1,212 and 1,070, respectively. Pooled T-SPOT.*TB* sensitivity was 81% (95% CI: 78%-84%; I^2=93.3%), similar to that of QFT-GIT sensitivity. Inclusion of papers not showing specificity (n=4) did not modify the above mentioned pooled T-SPOT.*TB* sensitivity (83%; 95% CI: 80%-85%; I^2=94.2%).

Pooled T-SPOT.*TB* specificity was 59% (95% CI: 56%-62%; I^2=84.5%) while the pooled diagnostic odds ratio was higher than that of QFT-GIT (18.86; 95% CI: 8.7–40.7; I^2=81.2%).

The median (IQR) proportion of T-SPOT.*TB* indeterminate results was 3.4% (5%) (5% (9.5%) in adults and 8% (2.5%) in children). A total of 227 children with tuberculosis and valid T-SPOT.*TB* results were evaluated. Mean (SD) sensitivity was 42.2% (11%) in all cases. Specificity was computed in only one study (74%).

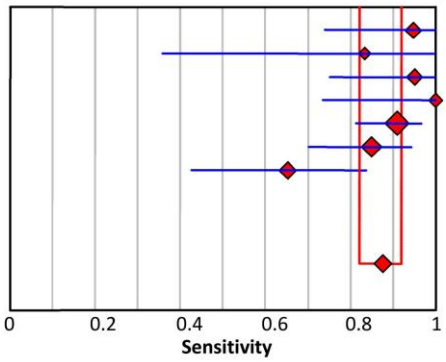
Characteristics of IGRAs performed on extrasanguinous samples

Nine out of the 27 articles (33.3%) described the diagnostic performance of IGRAs on extrasanguinous fluids: five on pleural fluid, three on bronchoalveolar lavage fluid, and one on ascitic fluid. None of these studies included children.

The total number of culture confirmed cases undergoing QFT-GIT was 64 with a median (IQR) number of 18 (24) per single study. Pooled sensitivity and specificity for all tuberculosis cases (culture confirmed and non-confirmed) were 48% (95% CI: 39%-58%; I^2=not assessed) and 82% (95% CI: 70%-91%; I^2=not assessed), respectively (see Figure 15 and Table 12). Pooled diagnostic odds ratio was 3.84 (95% CI: 1.73–8.5; I^2=not assessed). The proportion of QFT-GIT indeterminate results was very high, with a median (IQR) of 23.1% (40.1%).

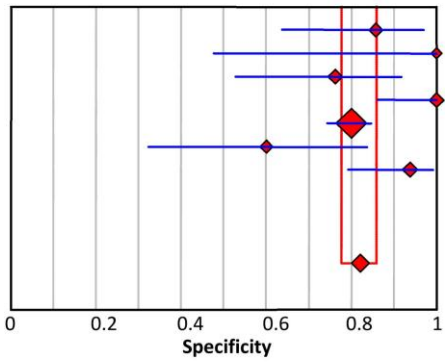
The total number of culture confirmed cases undergoing T-SPOT.*TB* was 128 with a median (IQR) number of 17 (26) in each study. Pooled sensitivity of the T-SPOT.*TB* for all tuberculosis cases (culture confirmed and non-confirmed) was 88% (95% CI: 82%-92%; I^2=57.9%), while pooled specificity was 82% (95% CI: 78%-86%; I^2=71.5%) (Figure 15, Table 12). Pooled diagnostic odds ratio, higher than that computed for QFT-GIT, was 35.83 (95% CI: 15.6–82.4; I^2=30.8%). The median (IQR) proportion of T-SPOT.*TB* indeterminate results was lower (5% (9.8%) than that of the QFT-GIT group.

Forest plots of sensitivity, specificity and diagnostic odds ratio are shown here only for the T-SPOT.*TB* performed on extrasanguinous samples as an example (Figure 15). A detailed description of all results and related graphical presentations are published in full in (SESTER et al., 2011). Sensitivity, specificity and diagnostic odds ratio of all assays as derived from the meta-analysis are summarized in Table 12.



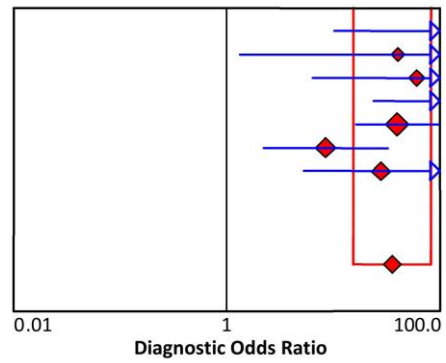
	Sensitivity	(95%CI)	Patients (n/n)
Lee LN et al [22]	0.95	(0.74-1.00)	18/19
Kim SH et al [20]	0.83	(0.36-1.00)	5/6
Losi M et al [23]	0.95	(0.75-1.00)	19/20
Jafari C et al [14]	1.00	(0.74-1.00)	12/12
Jafari C et al [15]	0.91	(0.81-0.97)	60/66
Dheda K et al [9]	0.85	(0.70-0.94)	34/40
Dheda K et al [8]	0.65	(0.43-0.84)	15/23

Pooled Sensitivity=0.88 (0.82-0.92)
Chi^2=14.25; df=6 (p=0.0270)
Inconsistency I^2=57.9%



	Specificity	(95%CI)	Patients (n/n)
Lee LN et al [22]	0.86	(0.64-0.97)	18/21
Kim SH et al [20]	1.00	(0.48-1.00)	5/5
Losi M et al [23]	0.76	(0.53-0.92)	16/21
Jafari C et al [14]	1.00	(0.86-1.00)	25/25
Jafari C et al [15]	0.80	(0.74-0.85)	199/249
Dheda K et al [9]	0.60	(0.32-0.84)	9/15
Dheda K et al [8]	0.94	(0.79-0.99)	30/32

Pooled Specificity=0.82 (0.78-0.86)
Chi^2=21.03; df=6 (p=0.0018)
Inconsistency I^2=71.5%



	Diagnostic OR	(95%CI)
Lee LN et al [22]	108.00	(10.24-1,138.79)
Kim SH et al [20]	40.33	(1.33-1,222.98)
Losi M et al [23]	60.80	(6.42-575.52)
Jafari C et al [14]	1,275.00	(23.87-68,092.84)
Jafari C et al [15]	39.80	(16.27-97.37)
Dheda K et al [9]	8.50	(2.21-32.76)
Dheda K et al [8]	28.13	(5.30-149.23)

Pooled Diagnostic Odds Ratio=35.83 (15.57-82.43)
Cochran-Q=8.67; df=6 (p=0.1932)
Inconsistency I^2=30.8%
Tau^2=0.3629

Figure 15. **Forest plots of sensitivity, specificity and diagnostic odds ratio of T-SPOT.*TB* performed on extrasanguinous samples. Sensitivity data represent pooled values that were computed on all TB cases (culture confirmed and non-confirmed cases). All studies reported data on both sensitivity and specificity. Numbers in brackets indicate the number of the study as listed in Annex 1.**

Table 12. **Sensitivity, specificity and diagnostic odds ratio of the different assays, for blood and extrasanguinous fluids.**

diagnostic assay / specimen		sensitivity	specificity	diagnostic odds ratio
TST		0.65	0.75	5.72
QFT-GIT	blood	0.80	0.79	11.47
	extrasanguinous	0.48	0.82	3.84
T-SPOT.*TB*	blood	0.81	0.59	18.86
	extrasanguinous	0.88	0.82	35.83

3.4 Research priorities are not EU-specific

To compile a roadmap with research priorities in the area of HIV/MTB co-infection, a total of 54 experts followerd the invitation to participate in the workshop, of which 30 (56%) originated from the field of HIV/AIDS and 24 (44%) had a TB background. The slight imbalance between HIV/AIDS and TB representatives resulted mainly from the full group of Russian TB experts (5 members) not showing up due to last minute organizational difficulties. As it was clear shortly before the workshop that only 7 of a possible 10 participants from India would be able to attend, two additional experts from Europe and one expert from the USA could be invited (Figure 16).

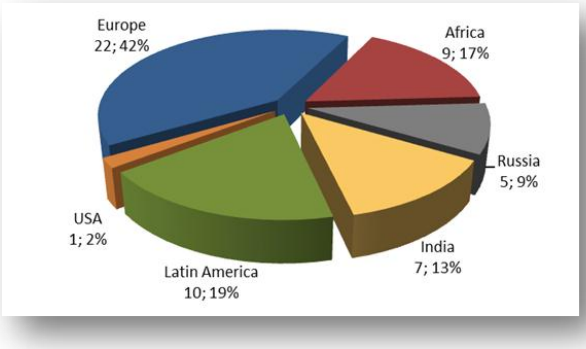


Figure 16. Workshop participants: Country distribution.

Participants came from a variety of different institutional backgrounds. The majority of the participants worked at research institutes, followed by university representatives (18/54, 34%), and members of non-governmental organizations (NGOs; 6/54, 11.1%) (Figure 17). NGOs that were represented at the workshop are 'Kuratorium Tuberkulose in der Welt e.V.', the 'European AIDS Treatment Group' (EATG), the US-based 'Treatment Action Group' (TAG), and the 'Joint United Nations Programme on HIV/AIDS' (UNAIDS; Reference Group on Estimates, Modelling and Projections). 8 of the 54 participants (15%) were clinicians.

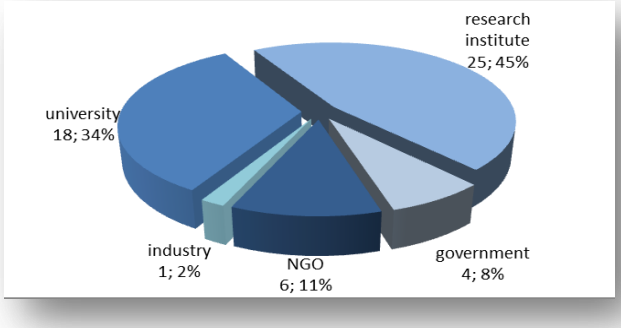


Figure 17. Workshop participants: Sector distribution.

The first workshop session comprised presentations of a baseline assessment for each of the participating regions, to provide all workshop participants with a specific insight into epidemiology and disease management standards in the participating regions. Each presentation was concluded by a summary of the key challenges for that particular region, followed by a personal 'favorite' or 'most burning' research question formulated by the presenter. While the overview of the epidemiological situation and disease management standards illustrated partially very distinct situations with great differences between the areas presented, and revealed that the EU27 - since highly heterogeneous - would require a more detailed and specific assessment, the suggested top priorities for further research were very similar between the different regions.

Selected key presentations on the current funding situation and ongoing international initiatives concluded the joint baseline assessment, based on which research priorities were then discussed and specified in three dedicated work groups, each comprising experts from both high and low burden regions. Although considered of high importance, no recommendations were developed with regards to development of vaccines and new drugs since these areas have been receiving by far the most funding throughout FP6 and the first three years of FP7 (LANG et al., 2010) or been part of the recent FP7 calls. Therefore large international consortia are already in place, pursuing highly promising research avenues. (Topics formulated by the experts are summarized below, and published in full in ALVAREZ et al., 2011).

3.4.1 Basic sciences

Given the rise of TB due to the HIV pandemic and the limited options to manage MDR- and XDR-TB, workshop participants agreed that there are strong accumulated needs to improve (i) the mechanistic understanding of TB pathogenesis especially in the context of an HIV infection, (ii) the therapy options and procedures, and (iii) the diagnostic tests predictive for latent and active TB infection.

Two topics in basic science were identified that deserve further attention by concerted research activities. The first suggested topic (see Table 13) aims to understand in greater detail the HIV/TB interactions within an infected host that lead to enhanced disease progression. The formation of MTB-induced granuloma, its role in MTB containment and release, and the influence of HIV and the antiviral immune response on these individual

functions need to be studied. A multidisciplinary approach, by clinicians, pathologists, immunologists, microbiologists and virologists, is needed to dissect the central cellular events. Increased knowledge of those mechanisms and regulatory pathways may allow the development of novel therapeutic strategies on how to prevent disease progression and contain MTB in the state of latency, or how to eliminate MTB that are kept under control by the immune system. The second priority topic suggests to transfer the significant progress which was made in understanding the development of HIV drug resistance (DEFORCHE et al., 2008; ROSEN-ZVI et al., 2008) to the TB field, and to combine it with TB-specific problems of drug resistance. By understanding drug resistance pathways, defining microbial costs associated with resistance, considering immune and nutritional status, and incorporating the knowledge on drug functions and interactions, one should be able predict optimal therapy strategies especially when the first line drugs are failing. A consortium incorporating clinicians, basic researchers, drug designers and bioinformaticians should be able to significantly improve HIV/TB management and help to reduce the frightening burden of multi-drug resistance worldwide.

Table 13. The two priority topics as recommended by the EUCO-Net experts for basic sciences.

	N°	topic title	aim	expected impact
basic sciences	1	**Understanding MTB pathogenesis in HIV infected individuals** by identification of microbial virulence and host factors	Understand microbial and host mechanisms contributing to different dynamics and clinical phenotypes of HIV-MTB co-infection. Means: highly standardized cohort studies, and the development and use of pre-clinical model systems (cell based or animal models).	Prediction of MTB progression across different stages of the HIV related disease. Precise knowledge on the pathogen interaction at the molecular and cellular level in the context of this polymicrobial disease may contribute to the identification of new therapeutic targets.
	2	**Personalized treatment approach for TB and HIV positive individuals** based on drug resistance and therapeutic drug monitoring	Optimally tailor treatment in HIV/MTB co-infected patients through therapeutic drug monitoring and the development of algorithms that predict microbial drug resistance. Based on the substantial knowledge on genotype, phenotype and clinical resistance in the context of HIV, a similar strategy will be developed for infection with MTB. Main goals include the identification of new drug resistance markers, mechanisms of drug resistance, and evolution of microbial fitness.	Personalized treatment shortening treatment duration and preventing therapy failure including resulting spread of drug resistant HIV/MTB variants.

3.4.2 Clinical sciences

The double infection with both MTB and HIV bears many challenges with regards to patient management. Multiple studies have shown a substantial survival benefit from ARV therapy, but many patients die of TB shortly after the initiation of antiretroviral therapy (LAWN et al., 2007), there are numerous interactions between anti-tuberculous and antiretroviral drugs (MA, LIENHARDT, 2009), and the pharmacokinetics of the drugs in children are incompletely understood (DONALD, DIACON, 2008). Advances are urgently needed as the lack of infection control measures in resource-poor settings, low TB treatment success rates in the large population of HIV-infected susceptible hosts, inadequate drug-sensitivity testing and overburdened MDR-TB treatment programs could combine to lead to a MDR-TB and XDR-TB epidemic of unmatched scale (ANDREWS et al., 2007). Key challenges in this research area are summarized in Table 14.

Table 14. Four priority topics as recommended by the EUCO-Net experts for clinical sciences.

	N°	topic title	aim	expected impact
clinical sciences	3	**TB control activities in HIV-positive individuals:** Cohort studies on HIV/AIDS	Integrate existing and future cohort studies involving HIV-infected patients living in areas with high incidence of TB. Main goals should be: improving diagnosis, prognosis, MTB infection control, prevention and treatment of TB, understanding the pathogenesis of this co-infection, especially immunopathogenesis, including IRIS and associated (predictive) factors increasing the understanding of causes of early mortality, outcomes of ART treatment in the context of hepatitis co-infection, and efficacy of TB preventive treatment.	A large cohort with harmonized and synchronized databases, sample collections and documentation from different health units in countries with high HIV/MTB co-infection will provide adequate statistical power for several retrospective and prospective analyses. Data using single cohorts or single countries can often not be evaluated and combining cohorts will be more cost effective than small and unpowered cohorts.
	4	**Treatment and Prevention of TB in HIV-positive individuals:** MultiCenter Clinical Trial Network on HIV/TB	Evaluate the interaction of ART and TB drugs, new TB drugs, and different regimens for prevention of TB in HIV-positive patients with or without ART. The trial will be designed to evaluate new treatment regimens for MDR and XDR-TB among HIV co-infected patients. This will also include the evaluation of pharmacokinetics and pharmaco-surveillance of anti TB treatment in the context of ART.	Increase the number of safe and potent drug regimens for prevention of TB in different settings of HIV infection according to different CD4 counts and/or different epidemiological situations. Develop alternative safe drugs for the treatment of MDR and XDR-TB for the treatment of HIV-infected patients.

	N°	topic title	aim	expected impact
	5	**Pharmacokinetics of antiretroviral and anti-MTB drugs in relation to clinical and pharmacogenomic**	Optimally define the drug dosages of anti-MTB and antiretroviral drugs in paediatric patients. Identify possible pharmacogenetic determinants of plasma drug levels with the final aim of individualizing drug dosage. Research	Significant improvement in the therapeutic management of children infected by HIV/MTB.
		characteristics in children with HIV/MTB co-infection	Should include the clinical follow-up for evaluation of treatment outcome in relation to plasma drug concentrations.	
	6	**Prevention of early mortality due to TB in HIV-positive individuals initiating ART** in geographic regions of high TB-incidence	Reduction in mortality due to TB in the first months following the initiation of ART in HIV-seropositive individuals. Possible geographic variations due to host and pathogen factors, different patterns of transmission as well as differences in treatment regimens should be elaborated. Main goals are the identification of risk factors for the TB-induced immune reconstitution inflammatory syndrome (IRIS) including markers of immune activation, genetic determinations and variables related to HIV and MTB-infection.	An increased knowledge on the effect of early ART will lead to a reduction in early mortality due to TB in HIV-infected individuals in countries of high TB incidence and will increase therapeutic options.

3.4.3 Diagnostics

Because TB is a treatable disease in principle, and HIV progression can largely be slowed down by antiretroviral drugs, rapid and efficient diagnosis of both infections is of prime importance in health care. While very sensitive and specific diagnostic assays are available to detect HIV infection, the diagnosis of LTBI or active TB disease is more demanding, and particularly challenging in HIV-infected individuals. The EUCO-Net experts therefore agreed that more sensitive, rapid point of care tests are urgently needed to improve case finding and access to treatment for co-infected individuals, and have summarized the most critical aspects in four topic suggestions as listed in Table 15.

Table 15. The four priority topics as recommended by the EUCO-Net experts for the area of diagnostics research.

	N°	topic title	aim	expected impact
Diagnostics	7	**Diagnosis of latent TB in HIV-infected patients**	Correctly identify patients latently infected with MTB, to predict their risk of progression, and to rule out active TB in both HIV-infected adult and paediatric patients. Key biomarkers should be identified, validated and implemented to be used in a clinical setting.	This monitoring technology should facilitate rapid targeting of chemoprophylaxis and therapy, and will enable to quantify the risk for progression and treatment success.

N°	topic title	aim	expected impact
8	**Diagnosis of active TB in HIV-infected patients**	Develop and validate improved rapid screening technologies for active TB in both HIV-infected adult and paediatric patients that are appropriate for use in TB-endemic countries and poor resource settings. Those tests could be based on a variety of biological samples.	Improved case finding, better treatment outcomes, reduced transmission, and better targeting of preventive therapy.
9	**Diagnosis of drug resistance in HIV-infected patients**	Develop and validate improved screening technologies to assess resistance to HIV and TB drugs in both HIV-infected adult and paediatric patients.	Prediction of treatment failure and quantitation of treatment efficacy. This will reduce transmission and improve patient care. Knowledge on drug resistance patterns will also inform infection control policies.
10	**Diagnosis of active TB in children in the context of HIV**	Develop and validate new approaches that identify active TB in the context of HIV in children.	Adequate treatment of TB in children and to the prevention of HIV/MTB-related childhood death.

3.4.4 Training and networking

As guidance documents that were developed by WHO and their partners (WORLD HEALTH ORGANIZATION, 2004; RAVIGLIONE, UPLEKAR, 2006) as the result of joint efforts by experts from both fields HIV/AIDS and TB are still far from being implemented into practice on a large scale, the EUCO-Net experts agreed that an improvement in integrating HIV and TB activities, training activities and increased networking are needed on three levels: At the programmatic level, where national TB and HIV/AIDS programmes have problems to transform formal contacts into effective collaboration, at the scientific level, where HIV/AIDS and TB communities are largely separate, including their meetings and conferences, and at the clinical level, where critical gaps between the two communities hamper the successful management of two diseases affecting one patient at the same time. It was agreed that dedicated co-infection platforms would be needed on the European level, to harmonize and streamline efforts among the European countries. While one platform should take into account the specific situation in Europe as described above, a global network would be needed in addition, to connect the European scientific community with all major endemic regions as well as leading global initiatives, allowing for concerted global action. Suggested initiatives are summarized in Table 16.

Table 16. The three priority topics recommended by the EUCO-Net experts for training and networking.

	N°	topic title	aim	expected impact
Training and Networking	11	**Training for translational research on HIV/TB**	In order to bridge the gaps between HIV and TB communities in the EU and the different high-burden regions, a comprehensive modular training approach is needed, targeted at clinicians, public health practitioners, researchers (incl. social researchers), patient community, policy makers, laboratory personnel, civil society groups, the media, and others. The modules should be developed using established means including task analysis, training needs, etc in order to be able to address the different needs of the target groups mentioned above, and should be easily applicable in different target regions.	Commonly agreed guidelines, identification of best practices in HIV and TB management and prevention in different sites & settings, improvement of management of HIV/TB co-infected patients, improved planning, implementation, and monitoring and evaluation of HIV/TB co-infection programmes, and creation of a common base for enhanced collaborative research efforts.
	12	**European TB Network of Excellence**	Integrate and synergize ongoing TB initiatives in Europe and help create a durable joint structure to promote stronger institutional integration between core partners of the network, with the aim of structuring and strengthening the European Research Area in the field.	Integrate and synergize ongoing TB initiatives in Europe and help create a durable joint structure to promote stronger institutional integration between core partners of the network, with the aim of structuring and strengthening the European Research Area in the field.
	13	**Global HIV/TB network**	Provide long-term sustainable interaction and coordination of efforts addressing HIV/TB co-infection. This will be achieved by detailed mapping of current networks of HIV, TB, and HIV/TB co-infection (what is there, what is lacking, how to synergize existing initiatives). Activities should include the establishment of a joint repository of knowledge, the creation of decision making models to aid national policy makers, the promotion of TB Infection control measures for HIV and TB infections, monitoring the implementation of existing HIV and TB guidelines, the implementation of a guideline quality control system, and assurance of fast integration of new developments into existing guidelines.	Integrate HIV and TB research in Europe, and connect the EU scientific community with all major endemic regions as well as leading global initiatives. It will build capacities and create a common knowledge base for coordinated action. A wide range of stakeholders, including especially patient organisations, should be represented

3.5 HIV/AIDS, TB, and co-infection in the global funding portfolio: distribution and trends

As a basis for the suggestion of future funding priorities in TB and HIV/MTB co-infection research, the current funding situation was analyzed, both on a global scale as well as specifically from a European perspective.

3.5.1 Global funding for HIV/AIDS & for TB research

The G-FINDER analysis of the latest available data on global R&D funding for HIV/AIDS (Figure 18) showed that by far the biggest share was going into vaccines research (65%), followed by microbicides (18%) and basic research (17%). Only 1% was dedicated to diagnostics research.

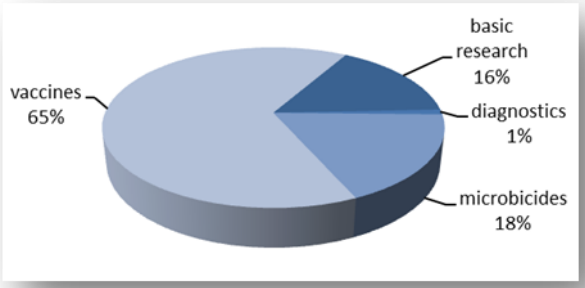


Figure 18. G-FINDER, HIV/AIDS funding 2007.

With regard to global TB funding, both analyses (G-FINDER and TAG, Figure 19) showed the majority of funding going to drugs (35% and 36%, respectively), with 21% and 22% going to vaccines, and 9%/10% supporting diagnostics research. In the G-FINDER report, drug research was closely followed by basic research with 32%, which was sub-divided into basic and operational research in the TAG report, receiving 20% and 7% respectively.

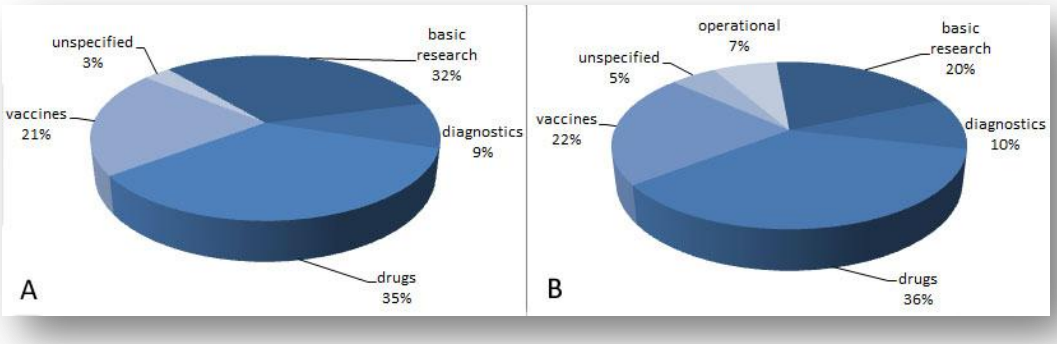


Figure 19. G-FINDER (A) and TAG (B) TB funding 2007.

3.5.2 HIV/AIDS and TB funding within FP7

As opposed to the allocation of global funding between different areas of research, the allocation of TB funding in FP6 and FP7 projects at the time of developing the Roadmap topics, as documented in the TAG reports 2005 – 2008, was clearly focusing on vaccines research, which received 42% of the total EU funding, followed by basic research (17%), drugs (16%), clinical research and epidemiology (13%) and diagnostics (12%) (Figure 20).

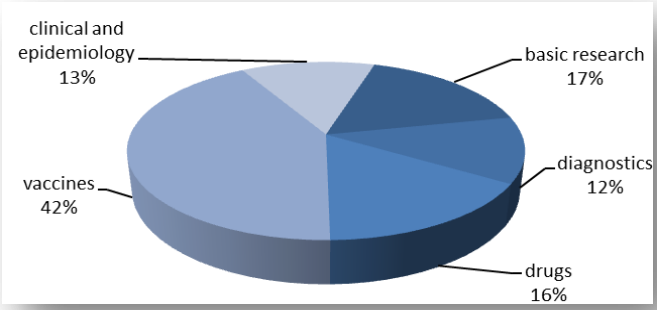


Figure 20. EU FP6/FP7 TB funding.

A graphical presentation of TB funding disbursement to the different disciplines in FP6 and FP7 over time shows that in the following years, the annual funding disbursements for vaccines research remained relatively stable, while funding for other fields increased. Vaccines research was temporarily surpassed by basic science research, which fell down to third rank again after vaccines and drug discovery more recently (Figure 21).

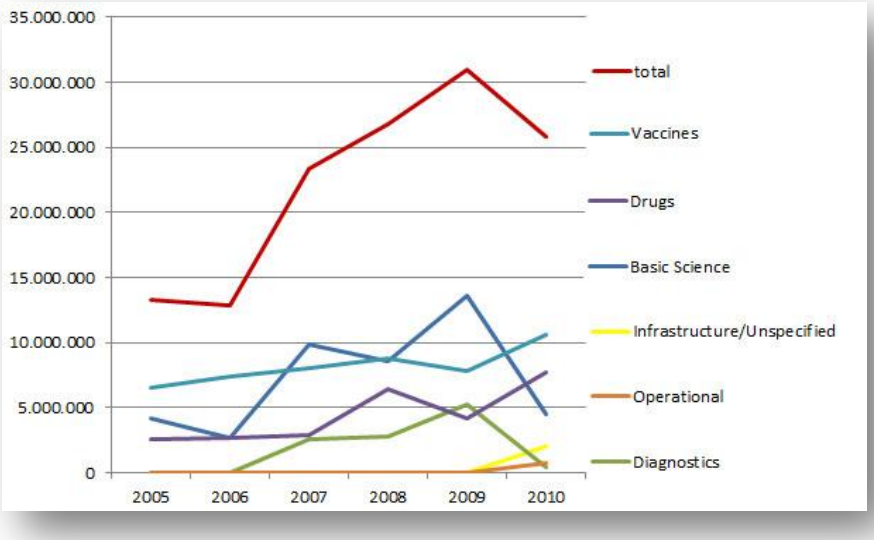


Figure 21. TAG: FP6/FP7 TB funding disbursement since 2005.

In May 2012, the EC had initiated funding for 25 projects that were dealing with either HIV/AIDS or TB alone (12 HIV/AIDS projects and 13 TB projects) under FP7. In addition, two projects were explicitly focusing on HIV/MTB co-infection issues. Based on information extracted from these projects, the calculated average monthly funding under FP7 for both diseases has been constantly increasing until April 2010, with average monthly TB research funding surpassing monthly AIDS research funding between September 2008 and February 2010. Since June 2010, funding for both has been falling again (Figure 22).

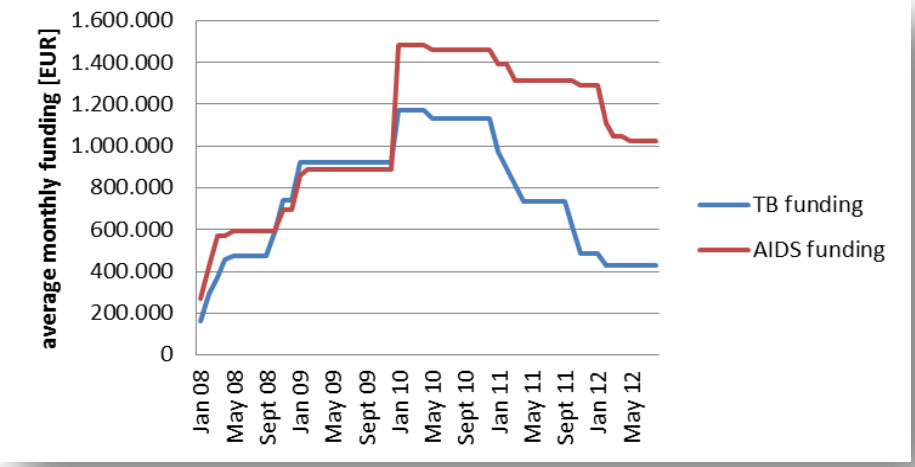


Figure 22. HIV/AIDS funding in FP7, excluding the HEALTH 2012 & 2013 calls (for which project contracts are not concluded yet).

However, the HEALTH-2012 call included several topics on infectious diseases research, and contract negotiations with the EC are currently ongoing, with new projects starting in late 2012 or early 2013. While 'HIV/AIDS, TB and Malaria' was closed in the HEALTH-2013 call, several topics offered the opportunity to include research on one or both of the diseases, e.g. 'HEALTH.2013.2.3.1-1: Drugs and vaccines for infections that have developed or are at the risk of developing significant anti-microbial resistance'. Thus, new funding is expected to go into HIV/AIDS and TB research from the last two HEALTH calls in FP7, while the exact amount cannot be estimated at the moment.

Of the total cumulative funding allocated to HIV/AIDS and TB projects to date, 82,234,186 EUR (62%) were invested in HIV/AIDS projects, while a maximum of 49,626,400 EUR (38%) EC contribution was allocated to TB projects, as shown in Figure 23.

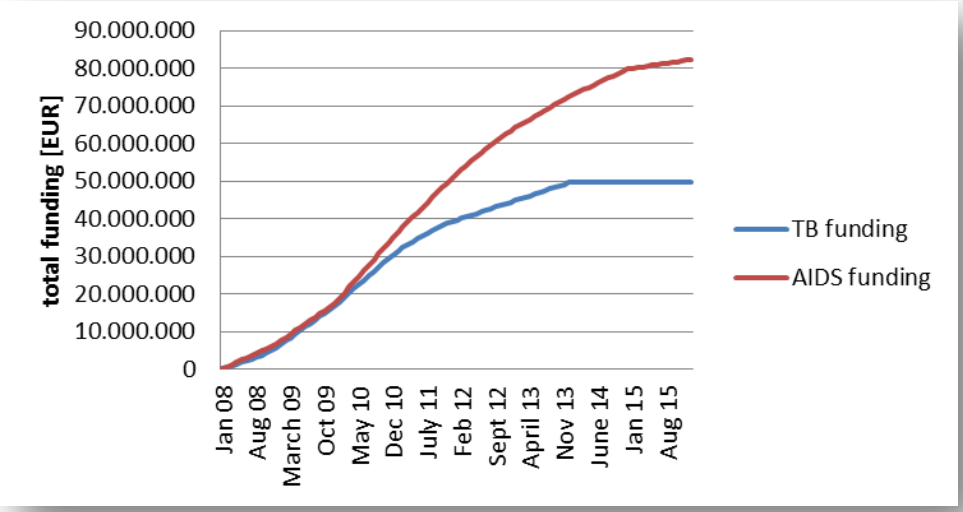


Figure 23. Cumulative HIV/AIDS & TB funding in FP7 up to 2012 HEALTH call.

The last characteristic that was systematically assessed for all FP7 projects to date was the distribution of partner countries, i.e. the countries in which the participating partner institutions are located. Figure 24 illustrates that of all countries, Germany, Italy and UK were most actively participating in TB projects. The most frequent EU partner countries involved in HIV/AIDS projects are Italy and France, followed by Germany, UK and Switzerland. Among countries with high TB prevalence, India, South Africa, Romania and Russia were involved in two or more projects.

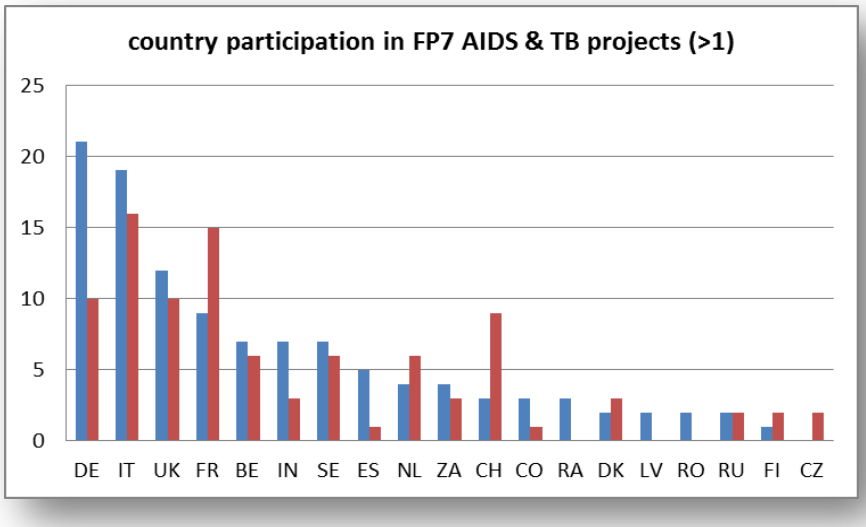


Figure 24. Countries involved in a minimum of 2 FP7 AIDS and/or TB projects, from left to right in descending order of number of TB projects.

3.6 Outreach

To increase public understanding of the severity and complexity of HIV/MTB co-infection and to support advocacy for dedicated and increased funding, a short film was produced and widely distributed among relevant stakeholders.

To communicate the complex immunological basics of HIV/MTB co-infection to the nonscientist public, the essence of the current available knowledge was distilled and translated into a **comic strip** (Figure 25 and Figure 26). The animated sequence starts with MTB infection, explains the mechanisms of the acquired immune response, and displays the formation of a granuloma as an effective means to keep the infection under control. HIV particles are shown as they attack the cells that are responsible for control of MTB, which leads to the disintegration of the granuloma and subsequently to the development of active TB and further spread of MTB.

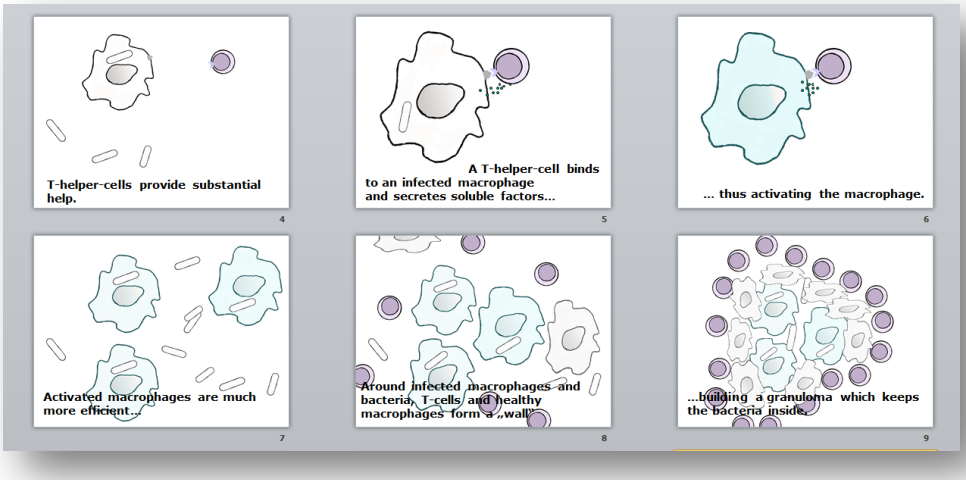


Figure 25. Animated presentation for the animation company: Macrophage activation and formation of the granuloma.

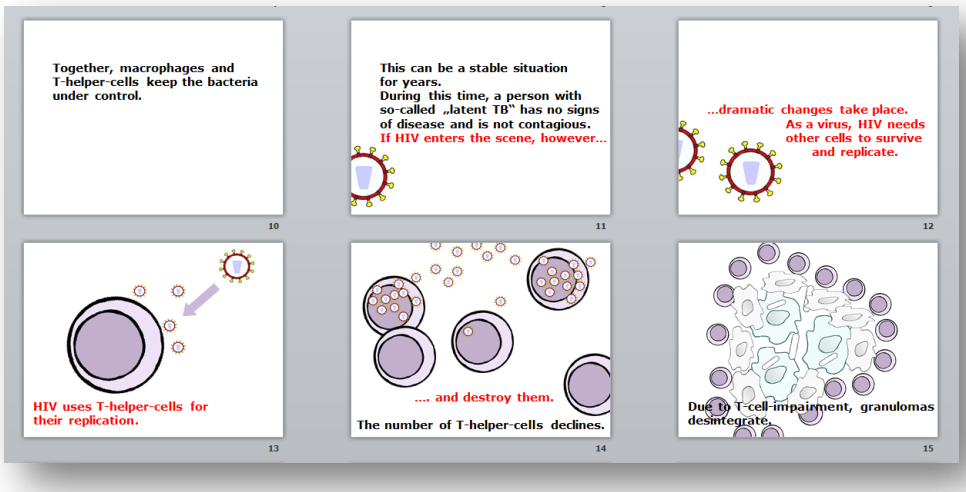


Figure 26. Animated presentation for the animation company: Effect of HIV infection on control of LTBI.

In addition to the basic information on immunological mechanisms of interaction between HIV and MTB and consequences for the infected host organism, the film shows patients affected by the two diseases, and portrays caregivers in a South African community clinic (Figure 27). HIV/AIDS and TB scientists from different high burden areas explain why particular challenges that require further research (Figure 28), and the EUCO-Net kickoff meeting in Brussels is shown as a starting point of the global EC-funded initiative (Figure 29).

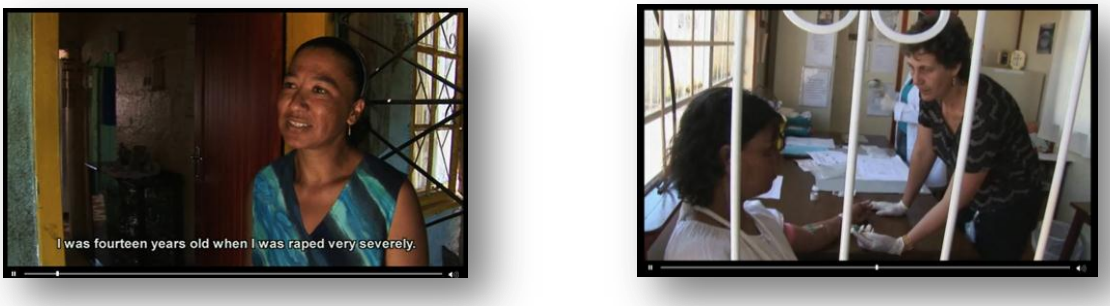


Figure 27. **A patient telling how the disease is affecting her life (left). Sister Susan van Zyl, taking care of a patient in the Uitsig Community Clinic (right).**

Figure 28. **AIDS expert M. Morgado, Brazil, and TB expert G. Walzl, South Africa, explaining challenges of HIV/MTB co-infection and outlining research questions that need to be addressed.**

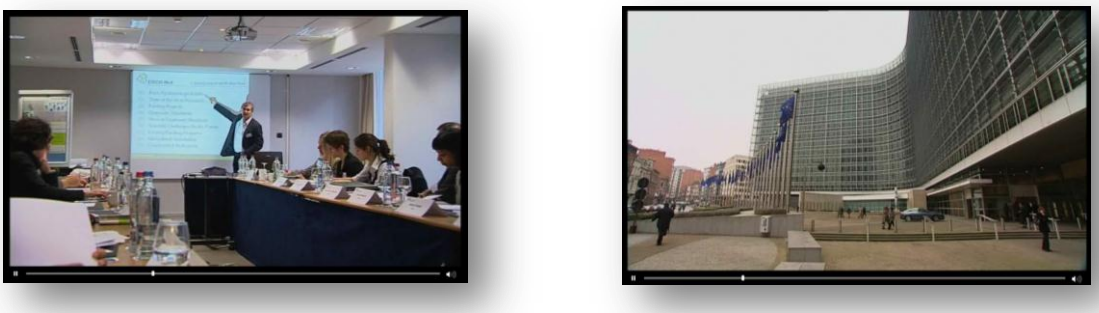


Figure 29. **HIV/AIDS and TB scientists from around the world coming together in Brussels, to initiate the 'European Network for global cooperation in the field of AIDS & TB' (EUCO-Net).**

The film was first presented at the Stellenbosch workshop in June 2009 and has been widely disseminated via public television, online, and in the form of hardcopies which were distributed to all workshop participants, as well as to selected **scientists, politicians, EC representatives and other stakeholders** at meetings, workshops, and scientific conferences.

Parts of the documentary were shown in the science programme "Nano" of the German **public television** channels ZDF & 3sat on World TB Day on March 24, 2009, accompanied by additional online articles and statistics about HIV/MTB co-infection. In May 2011, the film was presented in the framework of the **science awareness event** 'Lange Nacht der Wissenschaften' ('science night') at the medical campus of Saarland University.

Online dissemination started in November 2009 through several platforms. The film was placed on the public website of the EUCO-Net project (http://www.euco-net.eu) and published by the online service AlphaGalileo (http://www.alphagalileo.org), an EU online service for news releases and other information from science, health, technology, the arts, humanities, social sciences and business. Furthermore, the film was made available via the science video channel AthenaWeb (http://www.athenaweb.org), a video portal and audiovisual workstation comparable to YouTube, specifically directed towards science audiovisual professionals.

School children and university students were reached through presentations in seminars and workshops which included

- June 2009: Barcelona, Universitat Pompeu Fabra, Spain
- November 2009: Kopernikus Gymnasium Bargteheide, Germany
- June 2010: Gesamtschule Nohfelden-Türkismühle, Germany

Furthermore, the short film has been shown to **policy makers and programme representatives**, e.g. of the regional Ministry for Science and economics (Saarland Ministerium für Wissenschaft und Wirtschaft), the International Bureau of the German Ministry for Science and Education (BMBF), the German Science Foundation (DFG), or the Health Directorate of the European Commission.

4 Discussion

4.1 Epidemiological data

A comprehensive overview of the TB epidemiology (including MDR/XDR-TB) and HIV/MTB co-infection in all 27 EU member states was compiled as one of the elements to form a sound basis for the recommendation of priority issues for future research in Europe.

As a descriptive synthesis of disease surveillance data and epidemiological estimates that were published in part by the ECDC (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2010b), or that are accessible through the 'WHO's global TB database' (WORLD HEALTH ORGANIZATION, 2012b), it brings added value by providing a complete picture of the entire EU27 and presenting a detailed dataset for each of the 27 countries.

The following key messages could be derived from this compilation:

- TB surveillance data (including MDR/XDR-TB) in Europe are incomplete, illustrating serious shortfalls of the current disease surveillance system.
- HIV/MTB co-infection data are particularly scarce, so that conclusions could only be drawn from WHO estimates, which have to be interpreted with caution.
- The full set of TB epidemiological data compiled for this thesis reveals a diverse distribution pattern throughout Europe, with some Eastern European countries but also Spain, Portugal, and Austria ranking among the top five for one or more of the characteristics included in the overview.

Firstly, the collection of available data showed that even basic **TB surveillance data are incomplete** (GIEHL et al., 2011)**.** The full set of information that is needed to calculate prevalence, incidence and mortality (i.e. 'total TB cases at the time of data entry', 'new TB cases during year of reference', and 'TB-related deaths during the year of reference') were available for 22 of the 27 countries (81.5%). MDR-TB cases were reported by 26/27 (96.3%) of the EU countries, but data on XDR-TB were available for 11/27 countries (40.7%) only.

However, recent years have been characterized by a clear progress in TB monitoring in Europe. The 'Framework Action Plan to Fight TB in the European Union' (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2008) which was launched by the ECDC in 2008 made surveillance one of the eight strategic focus areas, and has paved the way to the

systematic monitoring of epidemiological and core operational indicators starting this year, in 2012, to be presented in annual TB surveillance and monitoring reports for Europe in the future (SANDGREN et al., 2012).

Secondly, the data set clearly illustrates that **HIV/MTB co-infection surveillance represents a particular challenge**, with many countries in Europe facing difficulties in collecting basic data on HIV/MTB co-infection (GIEHL et al., 2011). For the reference year 2008 for which the overview presented in this thesis has been compiled, co-infection data were available only for 14 of the 27 EU member states (51.9%). Again, the situation has since improved. A 2011 systematic review identified data on HIV co-infection among TB patients for 23 out of the 30 EU/EEA countries (76.7%) (PIMPIN et al., 2011), and a study assessing current practices in EU/EEA countries for monitoring HIV co-infection in TB surveillance systems, countries' current co-infection burden and associated clinical practice in the same year reported that the HIV status of patients was collected by TB surveillance systems in 18 out of 25, i.e. 72% of the countries that responded to the survey (KRUIJSHAAR et al., 2011). However, assessing the prevalence of HIV co-infection among TB cases even today is still compromised by suboptimal reporting in several countries, including within the European Union (PONTALI et al., 2011; ABUBAKAR et al., 2012), and five years after international recording and reporting guidelines were updated to include HIV status in TB registers and reports, many countries with paper-based systems are still unable to accurately report on treatment outcomes disaggregated by HIV status (WORLD HEALTH ORGANIZATION, 2011b). Of those countries that do report data on the association between HIV infection and TB, quality and completeness vary greatly, mainly due to differences in testing policies and data collection (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2010a), so that data have to be read and interpreted carefully.

Thirdly, based on the compiled set of information it can be concluded that **TB epidemiology in Europe follows a diverse distribution pattern**. In the reference year 2008, Romania was burdened by the highest prevalence and incidence, followed by Lithuania, Latvia, Bulgaria, Estonia, Portugal and Poland, all reporting >20 TB cases per 100,000 population (GIEHL et al., 2011). Thus, 7 of the 27 countries (25.9%) can be characterized as intermediate-to-high burden countries. Many but not all of these high prevalence countries lie in Eastern Europe. On the other hand, among the 20 EU countries (74.1%) that belong to the group of low TB-

prevalence countries (i.e. TB cases < 20 per 100,000), 6 countries had a prevalence of below 6 per 100,000. With regards to TB mortality, Austria ranked number four in Europe. The distribution of MDR-TB followed a similar pattern: While 4/5 (80%) of the countries with the highest MDR prevalence were Eastern European countries, Austria again ranked number five in Europe. When it comes to HIV/MTB co-infection, Spain and Portugal revealed a very high burden as compared to other European countries. 507 of the 1,402 total HIV/MTB co-infection cases (36.2%) in Europe were reported from Spain, while Portugal was leading in prevalence. These data confirm that 27 EU Member States are heterogeneous in terms of TB burden and characteristics of TB epidemiology, and that a reduction to 'WHO Europe Region' as applied by most epidemiology and surveillance reports up to that time is oversimplistic. Detailed reports specifically focusing on the TB epidemiology within Europe, as announced by the ECDC, will clearly facilitate informed decision-making in the future.

When interpreting the collected data, the following **limitations** need to be taken into account:

To deliver an overview that covers the countries and characteristics to be assessed in the most complete possible manner, **data from two different sources and to a very limited extent from different reference years were combined**. Data retrieved from different sources were routinely cross checked for consistency where values were included in both sources. As consistency was given in 100% of these cases, however, the combination of data seems appropriate. Data from different reference years are only indicated if no data for the standard reference year were available. In those cases, data indicated are clearly marked and correspond to the numbers for the closest available reference year.

Theoretically, data from TB surveillance systems that are linked to health systems of high coverage and performance may capture all (or almost all) TB cases, but so far no nationwide survey of TB incidence has been undertaken in any country (WORLD HEALTH ORGANIZATION, 2011b). In all surveillance data collections, patients are routinely 'missed' because i) they do not have physical or financial access to health care, ii) they seek care, but TB is not diagnosed, or iii) TB is diagnosed, but the case is not reported. **Only reported cases are documented in TB surveillance reports.** Thus, differences in reported case numbers do not necessarily only reflect differences in actual disease burden, but are strongly dependent

on the surveillance system in place in the respective country. This needs to be carefully taken into account when interpreting reported case numbers.

While the comparison of reported case numbers and WHO estimates revealed no striking differences with regards to overall TB prevalence or MDR-TB prevalence and incidence, the very **low number of countries that reported case numbers of HIV/MTB co-infection** represented such a clear limitation of the surveillance data collection that the overview was complemented by estimates for HIB/MTB co-infection.

According to these estimates, the highest burden of HIV/MTB co-infection lies in Southern Europe, with Spain bearing the highest case number, and Portugal and Austria leading in disease prevalence.

To assess the **exactness of the available HIV/MTB co-infection surveillance data**, the deviation between the number of reported cases and the value of the estimate was calculated. The comparison of actual surveillance data with given estimates is a useful indicator for the reliability of the reported case numbers (WORLD HEALTH ORGANIZATION, 2012a) and clearly illustrated that the reported case numbers need to be interpreted with caution, if at all. While Spain ranked number one in reported cases (507), the WHO estimate lies at 990 cases, thus nearly doubling the reported case number. The largest deviation between surveillance value and estimate is shown for Romania, with the estimated case number exceeding the reported case number of HIV/MTB co-infected patients by more than 200% (202 reported vs. 630 estimated cases). For the Czech Republic, on the contrary, the estimated case number lies more than 20% below the reported number. These examples illustrate that WHO **estimates need to be interpreted with caution**, and improvements in the surveillance system are urgently needed to deliver reliable information on the epidemiology HIV/MTB co-infection.

In conclusion, Europe is characterized by a diverse distribution pattern of TB epidemiology, with high and low burden countries, and different regional hotspots. Detailed and specific surveillance data are thus an important basis for informed decision making, both with regards to guideline development as well as to the development of research concepts. HIV/MTB co-infection data are particularly scarce, but monitoring efforts are clearly increasing. Thus, surveillance reports are significantly improving, both with regards to

reliability and completeness of data as well to the degree of detail and specific information presented on the 27 European Member States.

4.2 Diagnostics

In a systematic review and meta-analysis of published literature, evidence for the use of the commercially available IGRAs (T-SPOT.*TB* and QFT-GIT) and TST for diagnosing active disease was collected and analyzed (SESTER et al., 2011). Sensitivity and specificity for active TB was analysed in individuals with active TB and TB suspects with other diagnoses than TB, respectively. This represents a new approach of analysis as compared to previous studies that have used low-risk individuals as specificity controls (PAI, O'BRIEN, 2008; MORI, 2009; DIEL et al., 2010). Moreover, it adds a new element as compared to all previous studies by including IGRA performance in extrasanguinous fluids.

Three important messages could be derived from this analysis.

- Although the sensitivity of both IGRAs was higher than that of TST for the diagnosis of active TB, it was not high enough to recommend the use these assays as a single rule out test for TB.
- The specificity of IGRAs for the diagnosis of TB is much lower than previously reported (RICHELDI, 2006; PAI, O'BRIEN, 2008; MORI, 2009; DIEL et al., 2010) and inadequate to distinguish active TB from LTBI.
- Data suggest that the application of the T-SPOT.*TB* assay to extrasanguinous fluids is currently the best available immunodiagnostic method for the diagnosis of active TB.

In line with previous analyses, the **sensitivity** of both IGRAs for the diagnosis of active tuberculosis was higher than that of TST, with no difference of overall sensitivity between T-SPOT.*TB* and QFT-GIT. In general, however, the diagnostic sensitivity of IGRAs is too low to support their use as a rule-out test for TB, as the negative predictive value would not be sufficiently high (SESTER et al., 2011).

Pooled **specificities** of T-SPOT.*TB*, QFT-GIT and TST were as low as 59%, 79%, and 75%, respectively. Previous reports on the specificity of IGRAs for the diagnosis of tuberculosis that have exclusively used control groups with a low risk of MTB infection (PAI, O'BRIEN, 2008; MORI, 2009; DIEL et al., 2010), while this analysis included a study population which is much more similar to patients that would be tested in a routine clinical setting in Europe.

The lower specificity retrieved from the meta-analysis as compared to previous studies is owed to the fact that many of the individuals included here have positive IGRA or TST results, compatible with a diagnosis of LTBI. As this realistically reflects the actual situation in the setting where the tests are to be used, the low specificity obtained in this analysis emphasizes that no commercially available immunodiagnostic test is suitable to distinguish active TB from LTBI in clinical practice when using peripheral blood (SESTER et al., 2011).

The situation is different in **extrasanguinous samples**, where the pooled sensitivity was significantly higher for T-SPOT.*TB* (88%) compared to QFT-GIT (48%). In addition, the specificity of T-SPOT.*TB* in extrasanguinous samples was higher compared to that recorded in blood (82% vs. 59%), whereas specificity of QFT-GIT did not differ in the two compartments (79% vs. 82%). The median proportion of indeterminate results with T-SPOT.*TB* was similar in blood and extrasanguinous fluids, whereas QFT-GIT had more indeterminate results in extrasanguinous fluids. Thus, both the higher sensitivity and the lower proportion of indeterminate results indicate superiority of the T-SPOT.*TB* when analyzing extrasanguinous samples from patients with active TB (SESTER et al., 2011).

The urgent need for alternative diagnostic assays is emphasized by the fact that more than 50% of pulmonary TB cases have undetectable AFB in sputum smear examinations. While neither TST nor IGRAs are clinically relevant in AFB sputum smear-positive cases, a rapid TB case-detection must become possible in AFB sputum smear-negative patients as well, especially as more than 10% of secondary TB cases are contracted from persons with negative AFB sputum smears (TOSTMANN et al., 2008). In this situation, the T-SPOT.*TB* assay, applied directly on extrasanguinous fluids, could be an important improvement for a rapid decision to initiate anti-TB treatment in countries of low TB incidence. In such low-incidence settings, the required specimens are routinely collected in TB suspects with AFB sputum smear-negative TB, and the ELISPOT technology is available (LANGE, MORI, 2010).

One of the **limitations** of the meta-analysis lies in the considerable heterogeneity between studies. Furthermore, the number of studies including children was very limited. While immunocompromized patients were included in many of the studies, results were not reported in a stratified way which would allow the specific assessment of the effect of immunodeficiency on test accuracy. Thus, more studies are needed including paediatric patients as well as allowing a stratified analysis of the effect of immunosuppression.

In conclusion, the evidence collected shows that IGRAs have limited accuracy in diagnosing active TB. While the sensitivity of IGRAs performed on blood is higher than that of TST in patients with active TB, the low specificity illustrates a limited value of IGRAs to distinguish between latently infected individuals from patients with active disease. Conversely, as diagnostic accuracy is high when using T-SPOT.*TB* on extrasanguinous fluids, T-SPOT.*TB* is currently the best available immunological method to distinguish active TB from LTBI. However, the number of patients analysed so far is low, evaluation is relied on pooled data, and the application of IGRAs on extrasanguinous fluids for the diagnosis of active TB has not yet been evaluated by the regulatory authorities. Thus, definite evidence for the use of IGRAs from samples other than blood will require more, independent, and carefully designed prospective studies (SESTER et al., 2011).

The relevance of these results is emphasized by the fact that they have immediately been **taken up in European guidelines** (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2011) which state that i) IGRAs should not replace the existing standard diagnostic methods for the diagnosis of active TB, ii) a negative IGRA result does not exclude active TB disease, iii) as to the diagnosis of LTBI, IGRAs may be used in conjunction with an overall risk assessment in order to identify individuals for whom preventive treatment should be considered.

However, the inclusion of IGRAs in diagnostic guidelines on LTBI seems useful and reasonable only for Europe, where their use is current practice in many countries already, and where availability is not a limiting factor. Collected feedback from the performed survey on diagnostic standards and availability of tests shows that generally, a large variety of assays is available and routinely used in many EU countries, but not yet common practice in all. However, the abovementioned ECDC guidelines which have been in place since 2011 may help to further harmonize diagnostic standards in Europe. In resource-poor settings, the situation is completely different. While IGRAs were known in all countries included in the survey, they were used for research purposes only, and not at all part of clinical practice, neither for TB diagnostics in the clinic nor for contact tracing. This may in part be due to insufficient awareness, but is probably most likely caused by the limitation of resources. As compared to the TST, IGRAs have higher resource demands as they require laboratory access, trained personal, implementation of quality-assured procedures, and guaranteed

continuous access to reagents (EUROPEAN CENTRE FOR DISEASE PREVENTION AND CONTROL, 2011), which is just one of many challenges in high burden areas. Furthermore, the high prevalence of MTB in these settings would negatively impact sensitivity even further, to a point where IGRAs are not at all superior to the TST anymore, which is a second reason why their use cannot be recommended in international guidelines.

4.3 Funding

As a basis for the suggestion of future funding priorities in TB and HIV/MTB co-infection research, an overview of the global funding situation was compiled, and a detailed analysis of EU FP7 funding for HIV/AIDS and TB projects was performed. This should help to avoid duplication of efforts and maximize the impact of research funding.

The following observations drawn from this analysis were to be taken into account when formulating topic suggestions for future funding through the EU Framework Programmes:

- Global funding for HIV/AIDS strongly focuses on vaccine development, while the main share of TB research funding is invested in drug development.
- EU funding for HIV/AIDS and TB research was relatively balanced during the first half of FP7 and has only recently seen a relative increase of HIV/AIDS funding, while global R&D investment is strongly skewed towards HIV/AIDS.
- EU-funded TB projects have recently seen a significant decrease of funding disbursement for diagnostics and basic sciences.
- High burden countries are underrepresented in EU FP7 HIV/AIDS and TB projects.

The G-FINDER analysis showed that the **biggest share of global HIV/AIDS funding was invested in vaccines research** (65% of total HIV/AIDS funding), and both the G-FINDER and TAG reports confirmed that the **majority of TB research funding was going to drug development** (35% and 36%, respectively), followed by vaccines (21% and 22%), with only 9%/10% going to diagnostics research (GIEHL, 2011). This order fully reflects the R&D needs listed in the second Global Plan to Stop TB (RAVIGLIONE, 2006). Other important research areas for which funding is tracked specifically in the TAG report such as basic science or for operational research are not even mentioned in the Plan but their increasing importance has been emphasized (CHAISSON, HARRINGTON, 2009).

The most significant **limitation** of the data presented in large monitoring reports such as the ones provided by TAG and G-FINDER is survey non-completion. While the information provided by the main public funding agencies is considered to be nearly complete, private sector figures are described as under-reported due to non-participation of institutions, or provision of incomplete data. As an example, only 28 of a total of 150 SMEs (18.7%) that were identified to have neglected disease R&D activity provided data for the G-FINDER survey (MORAN et al., 2009a). Among those institutions which did report data, incorrect data entry or accidental omission of some grants is considered likely, though the impact of such mistakes is assumed to be minor. Also, the disaggregation of multi-disease grants in some cases may be difficult or impossible. Lastly, both monitoring reports include funding from e.g. the Global Fund, which is not really a research funding programme, but rather supports public health interventions. For all these reasons, figures indicated in the two monitoring reports need to be interpreted with caution, but still represent a valid and useful orientation with regards to global funding allocation to HIV/AIDS and TB research.

Global research spending is strongly skewed towards HIV/AIDS: While the annual number of deaths from TB and AIDS are very similar, the sizes of research budgets are disproportionate (HARRINGTON, 2010), with total reported HIV/AIDS funding amounting to 836.3 mio EUR as compared to 317.0 mio EUR dedicated to TB research according to the G-FINDER 2008 report (73% and 27%, respectively, of the total funding going into HIV/AIDS and TB research). As opposed to these global R&D investments, **funding in the ongoing European Framework Programme is more balanced**, with 12/25 funded research projects (48%) focusing on HIV/AIDS, and 13/25 projects (52%) dealing with TB research in May 2012, receiving a total cumulative funding of 82.2 mio EUR and 49.6 mio EUR (62% and 38%), respectively.

With regards to project participation, it is evident that **high burden countries are underrepresented in EU FP7 HIV/AIDS and TB projects.** Collaborative projects in FP6 and the first half of FP7 focused primarily on preclinical research, with very few phase I clinical trials, only some basic research, but increasing industrial development. The second half of FP7 has seen a strong increase in projects with clinical trials components, especially a large number of phase II clinical trials having started. As these phase II clinical trials have been introduced only recently, most of the projects submitted to the respective calls and selected

for funding are not under contract yet. Thus, participation of high prevalence countries is expected to clearly rise in the coming years.

In conclusion, particularly the detailed analysis of the funding portfolio of HIV/AIDS and TB research within FP7 allowed to develop topic suggestions which were complementary to ongoing initiatives and which would be able to fill identified funding gaps, such as the recent significant decrease of funding disbursement for diagnostics and basic sciences.

4.4 Research priorities

In the framework of the EUCO-Net project and building on a baseline assessment of the epidemiological situation as well as current funding portfolio, priority issues for future research in the field of HIV/MTB co-infection were discussed and defined jointly in an international and intersectoral approach (SESTER et al., 2010). The project has been funded by the Health Directorate of the European Commission and aimed to complement the results of the 'Challenges for the Future' conference on poverty-related diseases which was held in Brussels on Nov 13-14 2008 to develop views on how to focus PRD research under the new FP7 (GRYSEELS et al., 2009). Outcomes were published in the EUCO-Net Roadmap to help guide and stimulate funding. While several attempts to set priorities or define focus areas have been described for TB research in general, none of those specifically focused on HIV/MTB co-infection (RYLANCE et al., 2010).

The following main conclusions were drawn from this process:

- Interdisciplinary and intersectoral collaboration provide a clear added value.
- Basic sciences, clinical trials and diagnostics are key issues that need further funding.
- HIV/MTB co-infection requires dedicated platforms.

A unique characteristic of the EUCO-Net approach was its highly international, interdisciplinary and intersectoral design, and the inclusion of affected patients, which led to particularly fruitful discussions and useful exchange and broadening of knowledge, and resulted in a list of priority issues spanning the whole continuum from fundamental science to clinical research.

Four focus areas emerged from this process: basic sciences (two topic suggestions), clinical sciences (four topic suggestions), diagnostics (four topic suggestions), and training and networking (three topic suggestions).

Suggested **basic science focus topics** include research to elucidate MTB pathogenesis in HIV infected individuals through the identification of microbial virulence and host factors, and the development of a personalized treatment approach for co-infected patients based on drug resistance and therapeutic drug monitoring (ALVAREZ et al., 2011).

Clinical research priorities brought forward by the EUCO-Net experts include specific cohort studies to develop and assess TB control activities in HIV-positive individuals, and a multi-center clinical trial network on HIV/MTB co-infection to improve treatment and prevention of TB in HIV-positive individuals. Furthermore, it was suggested to elucidate the pharmacokinetics of antiretroviral and anti-MTB drugs in relation to clinical and pharmacogenomic characteristics in children with HIV/MTB co-infection, and to dedicate efforts to the prevention of early TB-related mortality in HIV-positive individuals initiating ART in geographic regions of high TB-incidence (ALVAREZ et al., 2011).

Furthermore, specific suggestions were formulated for **research into improved TB diagnosis** of in HIV-infected patients, both for active disease as well as for LTBI. Likewise, diagnosis of drug resistance in HIV-infected patients has been highlighted as a problem, and it has been recommended to fund activities that are specifically dedicated to improve diagnosis of active TB in HIV-positive children (ALVAREZ et al., 2011).

All experts agreed that in addition to the abovementioned research activities, **complementary initiatives** would be needed that provide training for translational research on HIV/MTB co-infection. A pan-European Network of Excellence for TB has been suggested as highly beneficial to widely diffuse knowledge and to harmonize efforts and research approaches throughout Europe, taking into account the diverse situation that the EU27 is confronted with. And lastly, the creation of sustainable global network specifically dedicated to HIV/MTB co-infection has been recommended (ALVAREZ et al., 2011).

These topics listed in the EUCO-Net Roadmap are published in (ALVAREZ et al., 2011), in line with the priorities identified in other initiatives described in the literature (CHURCHYARD et al., 2007; HESSELING et al., 2007; MARAIS et al., 2010; RYLANCE et al., 2010), and represent

an added value by providing detailed justifications and explicit descriptions of the expected impact and societal benefits that the recommended measures would bring about. In addition to scientific research questions that need to be addressed and concrete suggestions as to how these issues could be tackled, the group agreed that sustainable platforms for specific training and networking in the area of HIV/MTB co-infection would be needed, both specifically within Europe as well as in the form of a global initiative.

Appropriateness of selected method of defining research priorities: Several possible methods for the identification of focus areas are described in the literature, with consensus statements derived from expert group discussions being the most common approach. Although there are formal methods for prioritizing research, a systematic review evaluating 33 research agendas for tuberculosis published between 1998 and 2010 has shown that only few of the consensus groups used such methods (RYLANCE et al., 2010). Group discussion methods tend to provide large comprehensive lists of questions without prioritization and thus follow a similar approach as the EUCO-Net initiative. This approach has been described as useful for raising awareness and for funding in specific research domains, which were also the main declared goals of EUCO-Net. Furthermore, while according to the review the exact method used for the selection of focus topics is often not described, the process of topic selection and detailed description and justification of each of the priority issues has been clearly described for the EUCO-Net Roadmap.

In conclusion, the EUCO-Net approach is in line with recommendations and observations described in the literature, both with regards to methodology for the identification of focus topics as well as expert group composition. In an international, interdisciplinary and intersectoral approach, the project delivered a Roadmap on future HIV/MTB research, providing concrete suggestions of initiatives to funded covering basic research, clinical sciences, diagnostics and training and networking.

4.5 Outreach

To increase public awareness and understanding of HIV/MTB co-infection, and to maximize the exposure of key messages, a 12-min short film was produced and disseminated to a wide audience, including scientists, media representatives, policy- and decision makers, and the public at large.

In addition to the generation and application of new knowledge, its communication is one of the main traditional roles of science (LUBCHENCO, 1998), but scientific language is inherently academic and thus often inefficient in delivering relevant information to nonscientists (CRONIN, 2010).

To achieve these goals and to foster dialogue and debate, the EC has established an action plan to raise the awareness and knowledge of Europe's citizens regarding science issues (EUROPEAN COMMISSION, 2002) and requires FP7 project participants to communicate and engage with actors beyond the research community (EUROPEAN COMMISSION, 2004).

A Eurobarometer survey on 'Europeans, science and technology', requested by the EC Research Directorate-General, showed that 60% of the general public obtains its knowledge of science from television, and confirms the increasing role that the internet plays in informing public awareness and opinion (EUROPEAN COMMISSION, 2004). Thus, the audiovisual format has been chosen to achieve a timely, fast and effective transmission of key concepts, delivering accurate information in a condensed format which is still easy to understand by all relevant stakeholders, including non-scientists. While the extent of outreach cannot really be traced, and direct impact is difficult to measure, all individuals involved in the direct distribution of the film have reported that it has been taken up very positively and recommend this format for future science communication activities.

5 Cooperations

Chapter	Task	partner contribution	partner institution	contributors
3.2.2, 4.3	Systematic review and meta-analysis	Selection of studies to be included, data extraction & interpretation	TBNET	M. Sester, C. Lange, E. Girardi, GB Migliori, A. Bossink, K. Dheda, R. Diel, J. Dominguez, M. Lipman, J. Nemeth, P. Ravn, S. Winkler
3.2.2, 4.3	Systematic review and meta-analysis	Statistical analysis	TBNET	G. Sotgiu
3.3.3	Definition of priority topics, online follow-up	Programming of online platform	Progressima s.r.l.	A. Wagner, N. Weiler
3.5, 4.6	Short film production	Technical realization	Sichtzeit GmbH	T. Hillebrandt, R. Holz, D. Hunger, B. Wiggeshoff
3.5, 4.6	Short film production	Preparation of filming and local coordination in Cape Town	Stellenbosch University; Desmond Tutu TB Centre	A. Loxton; G. Black; S. van Zyl

6 Publications and Acknowledgement

6.1 Publications

1. **Management of tuberculosis in HIV infection: where T-cells matter.**
   Sester M, Giehl C, Sester U, Meyerhans A.
   Eur Respir J. 2010 Mar; 35(3):475-6. PMID: 20190327

2. **Challenges and perspectives for improved management of HIV/Mycobacterium tuberculosis co-infection.**
   Sester M, Giehl C, McNerney R, Kampmann B, Walzl G, Cuchí P, Wingfield C, Lange C, Migliori GB, Kritski AL, Meyerhans A; European Network for global cooperation in the field of AIDS and TB (EUCO-Net).
   Eur Respir J. 2010; 36(6):1242-7.

3. **Interferon-γ release assays for the diagnosis of active tuberculosis: a systematic review and meta-analysis.**
   Sester M, Sotgiu G, Lange C, Giehl C, Girardi E, Migliori GB, Bossink A, Dheda K, Diel R, Dominguez J, Lipman M, Nemeth J, Ravn P, Winkler S, Huitric E, Sandgren A, Manissero D.
   Eur Respir J. 2011; 37(1):100-11.

4. **Interferon- release assays for diagnosis of active pleural tuberculosis: a developing world perspective.** Lange C, Sester M, Sotgiu G, Giehl C, Girardi E, Bossink A, Dheda K, Diel R, Dominguez J, Lipman M, Migliori GB, Nemeth J, Ravn P, Winkler S, Huitric E, Sandgren A, Manissero D.
   Eur Respir J. 2011; 38: 747-748.

5. **World-Wide Challenges and Perspectives for Handling HIV/Mycobacterium tuberculosis Co-Infections.**
   Sester M, Giehl C, Kampmann B, Meyerhans A.
   The Open Infectious Diseases Journal, 2011, Volume 5 (Suppl 1-M1) 13.

6. **Research Priorities for HIV/M. tuberculosis Co-Infection**
   Alvarez MA, Arbelaez P, Bastos FI, Berkhout B, Bhattacharya B, Bocharov bG, Chereshnev V, Cuchí P, Däumer M, Demikhova O, Feldmann K, García LF, Giehl C, Gulalia

A, Kampmann B, Karamov E, Kebaabetswe P, Kiselev M, Knellwolf AL, Kritski AL, Lange C, López-Galíndez C, Makone A, Martin A, Mayanja-Kizza H, McNerney R, Meyerhans A, Migliori GB, Morgado MG, Nachega J, Ngwira B, Odermarsky M, Ota M, Palomino JC, Pfyffer GE, Preiser W, Ritacco V, Robledo J, Rodrigues C, Salomón H, Samper S, Sanchez J, Sester M, Seth P, Seti B, Sidorovich I, Singla R, Sonnier L, Torti C, Tortoli E, Vandevelde W, Vella S, Veloso V, von Briesen H, Walia K, Walzl G, Wingfield C.

The Open Infectious Diseases Journal, 2011, 5, (Suppl 1-M2) 14-20.

7. **The Situation of HIV/M. tuberculosis Co-Infection in Europe.**

   Giehl C, Basu Roy R, Knellwolf A.

   The Open Infectious Diseases Journal, 2011, 5, (Suppl 1-M3) 21-35.

8. **Supplementary Information on Global and European Funding on HIV/AIDS and M. tuberculosis/TB**

   Giehl C.

   The Open Infectious Diseases Journal, 2011, 5, (Suppl 1-M8) 89-90.

9. **TBNET - Collaborative research on tuberculosis in Europe**

   Giehl C, Lange C, Duarte R, Bothamley G, Gerlach C, Cirillo DM, Wagner D, Kampmann B, Goletti D, Jürs T, and Sester M.

   Eur J Microbiol Immunol, 2012. In press.

6.2 Invited oral presentations

12/2009 **Increasing Investment for TB Research and Development. EUCO-Net & FP7.**

TAG's Satellite Meeting at the 40th Union World Conference on Lung Health: 'Addressing Critical Challenges in TB/HIV Research and Program Implementation', Cancun, Mexico.

05/2010 **Priority areas for research and concerted actions: the EUCO-Net perspective.**

IV National TB Meeting/ I Brazilian STOP TP Partnership Forum, Rio de Janeiro, Brazil.

09/2010 **Research Priorities in HIV-TB: Results from the EUCO-Net project.**

Opening lecture to the presentation session 'Tuberculosis and HIV coinfection', 20th Annual Congress of the European Respiratory Society, Barcelona, Spain.

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8 Annexes

8.1 Annex I: List of 27 IGRA papers included in the meta-analysis

1. Baba K, Sornes S, Hoosen AA, Lekabe JM, Mpe MJ, Langeland N, Dyrhol-Riise AM. Evaluation of immune responses in HIV infected patients with pleural tuberculosis by the QuantiFERON TB-Gold interferon-gamma assay. BMC Infect Dis 2008: 8: 35.
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8.2 Annex II: List of extended group of EUCO-Net experts

Experts who participated in the Stellenbosch workshop, sorted by region.

EUROPE	**AIDS**		
	Andreas Meyerhans	Saarland University	Germany
	Hagen von Briesen	Fraunhofer Institute for Biomedical Engineering, IBMT	Germany
	Stefano Vella	Istituto Superiore di Sanitá, ISS	Italy
	Laure Sonnier	European AIDS Treatment Group, EATG	Belgium
	Ben Berkhout	University of Amsterdam, Academic Medical Center	Netherlands
	Martin Däumer	Institute of Immunology and Genetics	Germany
	Anne-Laure Knellwolf	Istituto Superiore di Sanitá, ISS	Italy
	Cecilio Lopez-Galindez	Instituto de Salud Carlos III	Spain
	Carlo Torti	University of Brescia, School of Medicine	Italy
	Wim Vandevelde	European AIDS Treatment Group, EATG	Belgium
	Paloma Cuchi	UNAIDS	Switzerland
	TB		
	Martina Sester	Saarland University	Germany
	Juan Carlos Palomino	Institute of Tropical Medicine	Belgium
	Enrico Tortoli	Careggi University Hospital	Italy
	Knut Feldmann	Kuratorium Tuberkulose in der Welt e.V.	Germany
	Beate Kampmann	Imperial College London	UK
	Anandi Martin	Institute of Tropical Medicine	Belgium
	Ruth McNerney	London School of Hygiene & Tropical Medicine	UK
	Giovanni B. Migliori	Fondazione Salvatore Maugeri	Italy
	Christoph Lange	Research Center Borstel	Germany
	Gaby E. Pfyffer	Luzerne General Hospital	Switzerland
	Sofia Samper	Instituto Aragonés de Ciencias de la Salud (I+CS)	Spain

USA	**AIDS**		
	Claire Wingfield	Treatment Action Group	USA

AFRICA	**AIDS**		
	Wolfgang Preiser	Stellenbosch University	South Africa
	Andrew Charman	Sustainable Livelihood Consultants	South Africa
	Jean Nachega	Stellenbosch University	South Africa
	Kebaabetswe Poloko	BOTUSA (Botswana – USA)	Botswana
	TB		
	Gerhard Walzl	Stellenbosch University	South Africa
	Albert Makone	Community Working Group On Health, CWGH	Zimbabwe
	Harriet Mayanja-Kizza	College of Health Sciences, Makerere Univ.	Uganda
	Bagrey Ngwira	Karonga Prevention Study	Malawi
	Martin Ota	Medical Research Council	The Gambia

INDIA	**AIDS**		
	Pradeep Seth	Seth Research Foundation	India
	Akash Gulalia	BPS Women University	India
	Suniti Solomon	YR Gaitonde Centre for AIDS Research and Education	India
	Kamini Walia	Indian Council of Medical Research	India
	TB		
	Basudev Bhattacharya	Institute of Postgraduate Medical Education	India
	Camilla Rodrigues	Hinduja National Hospital	India
	Rupak Singla	L.R.S.Institute of TB & Respiratory Diseases	India

SOUTH AMERICA	**AIDS**		
	Mariza Morgado G.	Oswaldo Cruz Foundation – FIOCRUZ	Brazil
	Francisco Bastos	Oswaldo Cruz Foundation – FIOCRUZ	Brazil
	Horacio Salomón	Argentinian Nat'l Reference Center for AIDS	Argentina
	Jorge Sánchez	Asociación Civil Impacta, Salud y Educación	Peru
	Valdilea Veloso	Oswaldo Cruz Foundation – FIOCRUZ	Brazil
	TB		
	Luis F García	Universidad de Antioquia	Colombia
	Viviana Ritacco	Consejo Nacional de Investigaciones Científicas y Técnicas CONICET	Argentina
	María Patricia Arbeláez	Universidad de Antioquia	Colombia
	Afriano Kritski	Federal University Rio de Janeiro	Brazil
	Jaime Robledo	Corporación para Investigaciones Biológicas, CIB-UPB	Colombia

RUSSIA	**AIDS**		
	Eduard Karamov	D.I. Ivanovsky Institute of Virology, Russian Academy of Medical Sciences	Russia
	Gennady Bocharov	Institute of Numerical Mathematics, Russian Academy of Sciences, RAS	Russia
	Valery Chereshnev	Institute of Immunology & Physiology, RAS	Russia
	Mikhail Kiselev	Ministry of Health and Social Development of the Russian Federation	Russia
	Igor Sidorovich	SRC, Institute of Immunology	Russia

9 Curriculum Vitae

Personal Data

Name	Claudia Giehl, née Schacht
Address	Rosenstr. 1, 66111 Saarbrücken
Date & Place of Birth	12.12.1974, Saarbrücken

Education

07/1985-07/1994	Albertus Magnus Gymnasium, St. Ingbert (degree: Abitur)
10/1994-04/1998	Saarland University, Medicine
04/1998-06/2003	Saarland University, Translation & Interpreting (degree: Diploma)
Since 09/2008	Doctoral studies, Saarland University, Institute of Virology, Prof. A. Meyerhans (until 06/09) & Prof. M. Sester (since)

Employment History

01-12/2005	'Advanced Translation Research Center' (ATRC), Saarland University: Research Assistant
01-04/2006	International Office, Saarland University: Project Leader
04/2006 - 2009	European Project Office, Saarland University: Project Officer
Since 2007	Eurice GmbH: Programme Manager LifeSciences

Vocational Training

27.-30.03.2011	TBNET 'Tuberculosis Academy' (academy for residents, doctoral students and postdocs), St. Leonhard, Austria
14.-15.06.2012	Seminar 'Clinical Tuberculosis' (continuing medical training, recognized by the Medical Association Schleswig Holstein), Research Center Borstel, Germany