Rational use of immunodiagnostic tools for tuberculosis infection: guidelines and cost effectiveness studies

Massimo Amicosante[1,2], Massimo Ciccozzi[4], Roumiana Markova[3]

[1]Chair of Respiratory Medicine, Department of Internal Medicine, University of Rome "Tor Vergata", Rome, Italy;
[2]ProxAgen Ltd., Sofia, Bulgaria;
[3]Laboratory of Mediators of Inflammation and Immunity, Department of Immunology and Allergology, National Center of Infectious and Parasitic Diseases, Sofia, Bulgaria;
[4]Department of Infectious., Parasitic and Immunomediated Disease, Istituto Superiore di Sanità, Rome, Italy

SUMMARY

Tuberculosis (TB) remains a public health challenge and its control requires the use efficient diagnostic tools. *Mycobacterium tuberculosis* (MTB) elicits a strong immune response upon infection, a phenomenon measured by the old tuberculin skin test (TST). However, this test has many limitations and a high rate of positivity in BCG-vaccinated subjects.

Recent studies have identified several MTB-antigens for diagnostic use, including the ESAT-6 and CFP-10 proteins. Based on these antigens, one of the most significant developments in the diagnostic armamentarium for TB has been the assays based on IFN- determination (IGRAs). The assays stem from the principle that T-cells of infected individuals produce IFN-gamma when they re-encounter the MTB antigens in vitro and this can be measured by a conventional ELISA test. The evaluation of IGRAs in different clinical settings showed many advantages over TST. The worldwide diffusion of IGRAs has increased the knowledge on their clinical use and a number of guidelines have been devised for their application. The two-step approach (first using TST followed by IGRA for confirmation) is the most favored strategy for IGRA-use in the general population, while the use of IGRAs alone is suggested in particular clinical settings and/or patient groups. Even if these tests are still costly there are a number of cost effective advantages in the "targeted" use of IGRAs over the TST. The work we present summarises all these aspects.

KEY WORDS: Tuberculosis, IGRA, Immunodiagnosis, Latent infection, Guidelines

Received February 2, 2010 *Accepted* March 3, 2010

INTRODUCTION

Tuberculosis (TB) is a public health challenge of paramount importance (World Health Organization, 2006). According to the World Health Organization (WHO), nearly 2 billion people - one third of the world's population - have been exposed to the TB pathogen (WHO report, 2006). Annually, 8 million people become ill with TB, and 2 million people die from the disease worldwide (WHO report, 2006). The rise in HIV infection and the neglect of TB control programs have enabled a resurgence of TB (Iademarco *et al.*, 2003). These factors, together with the emergence of drug-resistant strains have also contributed to this new epidemic with, from 2000 to 2004, 20% of TB cases being resistant to standard treatments and 2% resistant to second-line drugs (Centers for Disease Control and Prevention, 2006).

The rate at which new TB cases occur varies widely, even in neighboring countries, apparently because of differences in health care systems (WHO report, 2007). Further, the incidence of TB varies with age. In countries with a high-medi-

Corresponding author
Dr. Massimo Amicosante
Chair of Respiratory Medicine
Department of Internal Medicine
University of Rome "Tor Vergata"
Via Montpellier, 1 - 00133 Rome (Italy)
E-mail: Massimo.amicosante@uniroma2.it
Massimo.amicosante@ncipd.org

um incidence, TB primarily affects adolescents and young adults (Iademarco *et al.*, 2003). However, in countries where TB has gone from high to low incidence, TB is mainly a disease of older people, or the immunocompromised (Iademarco *et al.*, 2003). Consequently, control of TB will require a multifaceted approach integrating efficient public health interventions with the discovery and use of new diagnostic tools, vaccines and drugs. In particular, as the disease is usually transmitted from people with active - not latent TB, the rapid identification of subjects with active TB together with the identification of infected subjects represent the main measures to control disease diffusion efficiently (WHO report, 2006).

Mycobacterium tuberculosis (MTB), the etiological agent of TB, elicits a strong immune response upon infection by stimulating both CD4+ and CD8+ T-cells as well other cells of the immune system, determining a strong type-1 proinflammatory-like response dominated by interferon (IFN)-gamma and tumor necrosis factor (TNF)-alpha (Gomez *et al.*, 2004). The overall response is at the basis of the so-called delayed-type hypersensitivity (DTH) caused by MTB antigens (Huebner *et al.,* 1993). This phenomenon has been used for more than a century to identify subjects infected by MTB by using the tuberculin skin test (TST) which attempts to measure cell-mediated immunity in the form of a DTH response to the most commonly used purified protein derivative (PPD) of tuberculin (Huebner *et al.,* 1993). However, the test has many limitations not least the high rate of positivity following vaccination with *M. bovis* BCG (Huebner *et al.,* 1993).

INTERFERON-GAMMA BASED DETERMINATIONS

In the last decade, extensive studies have shown that immunodominant antigens, such as the 6-kDa early secretory antigenic target (ESAT-6) and its homologues, are highly suitable for detecting infection. There is no cross-reaction with the BCG vaccine, since these antigens are absent in the BCG vaccine strains. By screening eluted fractions of antigens from *MTB* and *M. bovis* culture filtrates for recognition by T-cells from infected humans and cattle, respectively, Andersen and coworkers identified several low-molecular mass antigens that are major targets of cellular mediated immune responses (Sorensen *et al.,* 1995). Subtractive DNA hybridization of pathogenic *M. bovis* and BCG (Mahairas *et al.,* 1996) and comparative genome-wide DNA microarray analysis of *MTB* H37Rv and BCG (Behr *et al.,* 1999) identified several regions of difference, designated RD1 to RD16, between *MTB* and *M. bovis.* All represent segments that have been deleted from the *M. bovis* genome. RD1 was lost early during the process of *M. bovis* BCG attenuation and is therefore missing in all the daughter strains known today (Mahairas *et al.,* 1996). This region has been the subject of detailed studies and a number of antigens have recently been characterized as candidate antigens for diagnostic and vaccine development (Van Pinxteren *et al.,* 2000; Andersen *et al.* 2000; Brusasca *et al.,* 2001). Antigens, such as early secreted antigen target (ESAT-6) and culture filtrate protein (CFP-10), are located in this region and have already shown great potential for TB diagnosis (Ulrichs T *et al.*, 1998; Ravn *et al.,* 1999; Arend *et al.*, 2000; Brock *et al.,* 2001).

Based on these studies, one of the most significant developments in the diagnostic armamentarium for TB in the last hundred years seems to be the assays based on IFN-γ determination (IGRAs). The assays stem from the principle that T-cells of sensitized individuals produce IFN-gamma when they re-encounter the antigens of *MTB* (Tufariello *et al.,* 2003). IGRAs' clearest advantage is increased specificity for detection of *MTB* infection thanks to their utilisation of *MTB*-specific antigens encoded in the region of difference (RD)1, a genomic segment absent from the Bacille Calmette-Guérin (BCG) vaccine and most environmental mycobacteria. Recent evaluations showed that IFN- assays that use *MTB* RD1 antigens, such as ESAT6 and CFP10, may have advantages over tuberculin skin testing (Arend *et al.*, 2000; Brock *et al.,* 2001 Lalvani *et al.,* 2001).

IFN-gamma assays that are now commercially available are the original QuantiFERON-TB, and its enhanced versions QuantiFERON-TB Gold and QuantiFERON-TB Gold In-Tube assays (Cellestis International, Carnegie, Australia), and the enzyme-linked immunospot (ELISPOT) T SPOT-TB assay (Oxford Immunotec, Oxford, United Kingdom).

Guidelines on the use of IGRAs

There is growing interest in the use of IGRAs, although most countries continue to recommend and use TST. In the past 5 years, several guidelines and position statements have been published on the role and use of IGRAs, and even some of the countries have more than one guideline or statement (Pai *et al.,* 2009).

At the present moment guidelines or statements on IGRAs have been identified from 16 countries, of which 9 European. At the present, USA, Canada, UK, Japan, France, Spain, Italy, Germany, Switzerland, Australia, Netherlands, Denmark, Czech Republic, Slovak Republic, Korea and Norway have available guidelines (Pai *et al.*, 2009). However, IFN-gamma release assays are regularly used in many other countries some including European countries like Bulgaria, Turkey, Portugal, Romania and Finland, and some of these are developing guidelines such as Finland and Portugal (Pai *et al.,* 2009).

Three main approaches are outlined in the countries that have guidelines:

- TST must be replaced by IGRA (i.e. only IGRA), as in Germany (for subjects undergoing anti-TNF-alpha therapy), Switzerland (for subjects undergoing anti-TNF-alpha therapy) and Denmark (for subjects undergoing anti-TNF-alpha therapy, BCG-vaccinated subjects, adult TB-contacts).
- Either TST or IGRA can be used, as in USA, France, Australia (for refugees), Japan (QFT preferred in all groups except in children <5 years) and Denmark (child TB-contacts).
- Two-step approach with TST first, followed by IGRA. This approach used either to improve specificity or sensitivity is suggested in Canada, UK, Italy, Spain, Australia, Germany (for TB contacts), Switzerland (for TB-contacts), Netherlands (for TB-contacts and immigrants), Norway and South Korea (for TB-contacts).

However, some guidelines recommend more than one approach, depending on the risk group tested (e.g. contacts, immunocompromised, children, etc.).

United States

The first published IGRA Guidance was the Centers for Disease Control and Prevention (CDC) Guidelines 2003, QFT (CDC, 2003). The CDC has issued recommendations on using the QuantiFERON-TB (QFT) test for diagnosing latent tuberculosis infection (LTBI). This document provided guidance for public health officials, health-care providers and laboratorians with responsibility for TB control activities in the USA in their efforts to incorporate QFT testing for detecting and treating LTBI. Regardless of the test used to identify LTBI, testing should be primarily targeted at diagnosing infected patients who will benefit from treatment. These interim recommendations are intended to achieve a high rate of acceptance and completion of testing for LTBI among groups who have been identified for targeted testing. Testing programs using TST or QFT should only be implemented if plans are also in place for the necessary follow-up medical evaluation and treatment (e.g., chest radiograph or LTBI treatment) of persons who are diagnosed with LTBI and quality laboratory services are ensured.

The CDC Guidelines 2005 were published on May 2, 2005 (Mazurek *et al.,* 2005). CDC recommended that QFT-G may be used in all circumstances in which the TST is currently used, including contact investigations, evaluation of recent immigrants, and sequential testing surveillance programs for infection control (e.g., those for healthcare workers).

The Guidelines for Preventing the Transmission of *Mycobacterium tuberculosis* in Health-Care Settings were published in December 2005 (Jensen *et al.,* 2005). They state that the whole-blood interferon gamma release assay (IGRA), QuantiFERON®-TB Gold test (QFT-G) (Cellestis Limited, Carnegie, Victoria, Australia), is a Food and Drug Administration (FDA) - approved in vitro cytokine-based assay for cell-mediated immune reactivity to *MTB* and might be used instead of TST in TB screening programs for Health Care Workers (HCWs). This IGRA is an example of a blood assay for *MTB,* does not require two-step testing and is more specific than skin testing and is not expected to result in false-positive results in persons vaccinated with BCG.

The American Thoracic Society (AST)/CDC/IDSA Guidelines require symptom screening, chest x-ray, and a TST upon HIV diagnosis and repetition of TST at least yearly.

The more recent US NIH Guidelines state that either a TST or IGRA should be used. Despite the best intentions of these guidelines and recommendations, 2006 statistics show that less than

1% of the world's HIV-infected population is screened for TB, let alone LTBI.
In 2008, the Guidelines for the Use of Antiretroviral Agents in HIV-1-Infected Adults and Adolescents came out (Panel on Antiretroviral Guidelines for Adult and Adolescents. 2008). It is stated there that immune restoration as a result of antiretroviral therapy may be associated with conversion from a negative to a positive tuberculin skin test (TST) or IFN- release assay (IGRA) in response to *MTB*-specific proteins. It is recommended that TST or IGRA should be repeated in previously TST-negative or IGRA-negative individuals after initiation of antiretroviral therapy when the CD4 cell count exceeds 200 cells/mm^3. HIV-infected individuals found to have LTBI, defined as >5 mm skin test induration or positive IGRA with no prior treatment for LTBI and after appropriate evaluation to rule out active TB disease and no prior treatment of LTBI, should commence treatment with isoniazid for 6 to 9 months.
The CDC/NIH/IDSA Guidelines 2009 for prevention and treatment of Opportunistic infections in HIV-infected adults and adolescents were published on April 10, 2009 (CDC/NIH/IDSA Guidelines 2009). Evidence suggests that the IGRAs have more consistent and higher specificity (92%-97%) than TST (56%-95%), better correlation with surrogate measures of exposure to *MTB*, and less cross reactivity because of Bacillus Calmette-Guérin (BCG) vaccination or other nontuberculous mycobacteria exposure than the TST. Three IGRAs are FDA-approved and available in the United States, the QuantiFERON®-TB Gold, the QuantiFERON®-TB Gold In-Tube (Cellestis Limited), and the T-SPOT™.TB test (Oxford Immunotec). For both the TST and IGRAs, however, HIV- related immunosuppression might be associated with false-negative results. The frequency of false-negative and indeterminate IGRA results increases with advancing immunodeficiency. Results from comparative studies of TST and IGRAs in HIV-infected patients indicate that concordance between the tests is not complete. The TST remains useful for diagnosing LTBI, particularly for patients who have not been vaccinated for BCG and in settings with cost constraints. The optimal application of IGRAs in HIV-infected persons will be better defined when the results of ongoing studies become available. IGRAs might be used in combination with TST to improve sensitivity and specificity for detection of LTBI. Given the high risk for progression to active disease in HIV-infected persons, any HIV-infected person with reactivity on any of the current LTBI diagnostic tests should be considered infected with MTB.
In the USA AAP Red Book 2009 (American Academy of Pediatrics. 2009, 28th Edition) it is stated that at this time neither IGRA not the TST can be considered a "gold standard" for diagnosis of LTBI. Current recommendations for the use of IGRAs in children are as follows:
For immune competent children 5 years of age and older, IGRA can be used in place of a TST to confirm cases of tuberculosis or cases of LTBI and likely will yield fewer false-positive results.
Children with a positive result from IGRA should be considered infected with *MTB* complex. A negative IGRA result cannot universally be interpreted as absence of infection.
Because of their higher specificity and lack of cross-reactions with BCG, IGRAs may be useful in children who have received BCG vaccine. IGRAs may be useful to determine whether a BCG-immunized child with a reactive TST more likely has a false-positive TST reaction caused by the BCG.
IGRAs cannot be recommended routinely for use in children younger than 5 years of age or for immune-compromised children of any age because of a lack of published data about their utility with these groups.
Indeterminate IGRA results do not exclude tuberculosis infection and should not be used to make clinical decisions.
The CDC Guidelines 2009, QFT-GIT/TSPOT.TB, will soon be released comparing the two ex vivo immunodiagnostic tests for TB. At the 2nd Global Symposium on IGRAs, Dr Ken Castro, Director of the CDC's Division of Tuberculosis Elimination, presented IGRAs as the 'preferred' test in BCG-vaccinated individuals and some other populations. The approach is a significant change from the previous guidelines and now considers the wide prevalence of BCG-vaccinated populations within the U.S.

Canada
The first Canadian CTC Guidelines on IGRAs, published in 2007, stated that:

- Two-step approach in contacts, immunocompromised is recommended.
- Not recommended in children.
- Not recommended for active TB and serial testing.
- An updated version of the CTC guidelines was published in 2008 (Canadian Tuberculosis Committee. 2008):
- Not recommended for active TB in adults.
- Can be used as a supplementary aid in children with suspected TB.
- IGRAs may be used as a confirmatory test for a positive TST in contacts (adult or child).
- IGRA may be performed in TST- positive, immunocompetent adults and children who are at relatively low risk of being infected and of progressing to active disease.
- In an immunocompromised person (adult or child), the TST should be the initial test. However, a clinician still concerned about the possibility of LTBI may perform an IGRA.
- Not recommended for serial testing of HCWs. IGRAs may be used as a confirmatory test if a false-positive TST is suspected in a low-risk HCW or prison staff /employee or inmate.

Australia

The Australian NTAC Guidelines were published in 2007 (Australian Government, October 2007) and the main points are:

- Currently TST remains the preferred method of screening for LTBI pending further evaluation.
- TST and IGRAs have almost no place in the diagnosis of active TB disease.
- State-based TB services should be encouraged to participate in the evaluation of the role of IGRAs for the investigation of LTBI.
- IGRAs may be used as a supplementary test in individualized clinical assessment for LTBI where increased specificity is valuable in reducing the confounding effect from prior BCG vaccination or prior exposure to non-tuberculous mycobacteria.

In 2009 the Australian Society for Infectious Diseases published its guidelines (Australian Society for Infectious Diseases Refugee Health Guidelines Working Group, 2009) and it states that, "With the exception of those with documented past TB disease, all newly arrived refugees, including children, should be assessed for LTBI, with the following plan:

- Testing is performed with the intention to treat.
- Either a Mantoux test or IGRA may be used for screening.
- Refer those with a positive Mantoux test or a positive IGRA test to the local TB services, for exclusion of active TB infection and consideration of treatment of LTBI.
- A Mantoux greater than 10 mm in adults and children of ≥5 years of age and a Mantoux of 5mm in those younger than 5 years or those who are HIV-infected are considered positive.
- Refugees known to be HIV-infected should have a two-step Mantoux test. In the event that the second test remains <5 mm, specialist advice should be sought from TB/HIV services.
- TB (active disease or latent infection) should be managed by clinicians experienced in doing so as part of a centralized, coordinated TB service.

Japan

In 2006, in Japan was published the JST Guidelines, regarding the use of QFT-2G (Committee of Prevention, Japanese Society of Tuberculosis. 2006). These are the main recommendations:

- Children <5 years:
- QFT not recommended [TST preferred] for LTBI.
- QFT can be used as adjunct for active TB.

Contacts:

- Less than 5 years old, TST is preferred over QFT.
- From 5 to 12 years old, Use QFT with considering use of TST together. The QFT results should be interpreted carefully.
- From 12 to 18 years, QFT is preferred over TST where QFT test is available. Use TST if necessary.
- From 18 to 49 years old, QFT is preferred over TST.
- Over 50 years old, Limited use of QFT or TST.

Health CareWorkers:

- QFT preferred over TST.

Active TB:

- Adjunct (supporting) evidence.

High risk groups (diabetes, steroids, TNF-alpha blockers):

- QFT is preferred and can be used for deciding on LTBI treatment.

Even when QFT is preferred:

- TST may be done first, followed by QFT in

TST+, in order to be cost effective (especially in case of mass screening).

In 2009 the QuantiFERON-TB Gold In - Tube assay was approved in Japan.

South Korea

The KCDC Guidelines in South Korea came out in 2009. They state that:

- IGRA is required to confirm TB infection in contacts over 6 years old with a positive TST (5mm in BCG unvaccinated and 10mm in BCG vaccinated.
- In contacts with negative TST results, IGRA can be performed based on the attending doctor's clinical decision.
- Not for active TB or serial testing.

In Europe there are several countries that released Guidelines on LTBI diagnosis during the last 3 to 4 years.

United Kingdom

On March 22, 2006, The National Institute for Health and Clinical Excellence (NICE) published the Guidelines for TB control in England and Wales (NICE. 2006).

The NICE Guidelines recommend:

- Use IGRAs as the front line test for latent TB infection in preference to the tuberculin skin test (TST) where the skin test may be "less reliable" including all immunocompromised patients.
- Use IGRAs as a secondary, confirmatory test in all cases when the TST is positive. The IGRA is used as a means of screening out TST false positives.
- IGRAs also have a role to play in the diagnosis of TB disease especially in non-pulmonary TB and as a rule-out test in TB suspects.

In October 2007, The Health Protection Agency published a position statement on the use of IGRA tests for TB, providing an update on the NICE Guidelines (Health Protection Agency. 2007). The main points of this statement are:

Active disease:

- IGRAs may be used when it has not been possible to confirm a diagnosis by culture and when radiological and histopathological evidence is lacking.

Latent Infection:

- In LTBI IGRAs are at least as sensitive at the TST and in BCG vaccinated populations are more specific.
- TST should be carried out first and those that are positive should be considered for IGRA testing if available.
- This would also apply to new entrant screening.

IGRAs should be the only test used in the following situations:

- Where TST may be falsely negative due to immunosuppression.
- When screening a large number of people as part of a public health investigations since repeated visits would be impractical.

Health care worker screening:

- New health care workers should be tested with IGRAs as they may come into contact with immunosuppressed patients and because of the logistical simplicity of the tests.

Pre-TNF- alpha screening:

- IGRAs may be a suitable alternative in BCG vaccinated subjects.

In July 2009, Guidelines for the Diagnosis and treatment of tuberculosis of the central nervous system in adults and children were published (Thwaites *et al.*, 2009). The recommendations made are that CSF adenosine deaminase activity is not recommended as a routine diagnostic test for CNS tuberculosis. The tuberculin skin test and IGRAs may provide indication of previous tuberculosis infection and are probably most useful in young children, but results need to be interpreted cautiously as neither is sufficiently sensitive nor specific to diagnose active disease. Currently, IGRAs are only licensed for the diagnosis of latent tuberculosis and cannot be recommended for the diagnosis of active CNS disease.

Switzerland

In November 2005 the Swiss Lung Association released recommendations for the diagnosis of TB infection in contact investigations using blood tests in Bulletin 45/10 of the Office Federal de la Sante Publique (Swiss Lung League 2005). The main points are:

- Confirm a positive Mantoux tests with an IGRA.
- Use only an IGRA in immunocompromised subjects.
- Children are excluded from being tested with IGRAs.

In December 2007 the following guidelines were released for the use of IGRAs to screen patients prior to administration of anti-TNF alpha therapies (Beglinger *et al.,* 2007). All patients should be screened for LTBI before being given anti-TNF alpha therapies:

- Screening should be based on history, chest x-ray and IGRA (TST is no longer recommended).
- Preventive treatment should be given where LTBI is suspected as a result of:
  1. Positive IGRA.
  2. Abnormal x-ray suggesting TB which was not adequately treated.
  3. History of significant prior exposure.

Italy

Recommendations for the identification of LTBI were released jointly by the Associazione di Microbiologia Clinica Italiana (AMCLI) and the Federazione Italiana per le Malattie Polmonari Sociali e la Tubercolosi (FIMPST) in May 2006 (AIPO, SIMeR. 2006). The main points are:

- Mantoux testing should be carried out and where positive an IGRA should be performed.
- In subjects with an expected TST positivity rate of 40% or more, and in immunosuppressed patients an IGRA should be carried out without a prior TST.
- IGRAs may be used along with other tests in the diagnosis of active disease.

In 2009 came out a draft awaiting approval by the Italian Ministry of Health (Girardi *et al.*, 2009) which states that:

- TST is the standard test for LTBI diagnosis, there is not enough evidence to recommend complete replacement of TST with IGRAs.
- IGRAs are recommended to confirm LTBI diagnosis in TST+ BCG- vaccinated individuals.
- IGRAs are recommended to diagnose LTBI in TST- HIV+ or other immunosuppressed individuals.

The Netherlands

Dutch Guidelines for contact tracing and screening were released in 2007 (Commissie voor Praktische Tuberculosebestrijding. 2007). The main points are:

- Contact investigations, TST is the initial test, if 5 mm or more followed by IGRA.
- Immigrant screening of children 4 weeks to 12 years old, initially TST, if 5 mm or more, then IGRA.
- Repeated screening of risk groups (e.g. occupational), either TST or IGRA as initial test.
- For detecting LTBI before start of iatrogenic immune suppression (e.g. anti-TNF-alpha therapy), TST and clinical information only.
- In subjects where the Mantoux "may be less reliable" perform an IGRA without a prior Mantoux.
- IGRAs can be used in place of a Mantoux in the work up for active disease diagnosis.

France

The Haute Autorite de Sante (HAS) issued preliminary guidelines in December 2006 suggesting that IGRA tests should be used in the following settings (Test de détection de la production d'interféron g pour le diagnostic des infections tuberculeuses (2006) (Haute Authorité de Santé. 2006):

- Contact tracing in subjects older than 15 years.

Health care workers where a TST may not be reliable.

- To assist in the diagnosis of active disease, particularly non-pulmonary.
- Pre-TNF alpha therapy screening.

Ireland

Draft guidelines published in July 2008 proposed that IGRAs can be used in the following settings (Immunisation Guidelines for Ireland. 2008 Edition).

- Contact tracing (in conjunction with a TST).
- In certain circumstances IGRAs, if available can be considered as the sole test for LTBI:
- Where the TST may be falsely negative due to immunosuppression.
- When screening large numbers of individuals as part of a public health investigation.
- Pre-employment screening of healthcare workers.
- For individuals, commencing immunosuppressive therapy e.g. TNF- antagonists.

Germany

In 2007 Recommendations for contact tracing in tuberculosis were published (Diel *et al.,* 2007) and in July 2009 New TB testing recommendations for autoimmune diseases came out (Diel *et al.,* 2009). It is stated that due to the increased risk of

tuberculosis (TB) under treatment with TNF-alpha inhibitors for rheumatoid arthritis and other autoimmune diseases, precautionary measures are required before initiating TNF-alpha inhibitor therapy. Patients should have active TB ruled out and screening for latent TB infection should be performed. The screening should include chest X-ray, complete medical history, and the administration of a highly specific interferon- release assay (IGRA). (In the future, the reimbursement of IGRA tests under an analogue procedure code is expected to be formalized by the application of a code specific to the TB IGRA procedure.) As tuberculin skin test (TST) results can be expected to be either false-positive or false-negative in these patients, the TST, as commonly performed in the past, is recommended only in exceptional situations.

Spain
The Recommendations of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR) published in 2008 (SEPAR. 2008) state that IGRA tests could be performed in immunocompromised individuals and children and prior to tuberculosis vaccination. They are not recommended in active TB and serial testing.

Denmark
Draft Guidelines on the use of IGRAs are proposed for discussion in 2009.
TB screening before anti TNF-alpha therapy:
- IGRA is preferred. TST when IGRA is not available.
- Exposure/contact screening:
- IGRA if BCG vaccinated/adult.
- Either TST or IGRA in children and unvaccinated young people.
- HIV, no specific guidelines.

Norway
The Norwegian Guidelines came out in 2007. The recommendations are as follows:
- TST as a first test and then IGRA as a supplementary test for all TST positives >6 mm.
- Among IGRAs,QFT is recommended as the primary test. If the mitogen control fails or the patient is severely immunosuppressedT-SPOT-TB is recommended as a secondary test.
- Referral to an infectious disease specialist or lung physician is recommended for all IGRA positives and also for TST >15 mm/IGRA negative. Result of chest X-ray, symptoms, previous history of TB, immune status is also assessed and part of the decision of referral and prescription of preventive treatment.

Czech Republic
The Recommendation of the Czech Thoracic Society for QuantiFERON-TB Gold test came out in December 2005. According to it the QuantiFERON-TB Gold test should be used in following situations:
- Differential diagnosis of active TB.
- Detection of TB infection in close contacts.
- Screening for LTBI in high risk groups.
- Before and during biological (anti- TNF-alpha) treatment.

Slovak Republic
A Draft of the Slovak Guidelines from 2008 on the use of IGRAs is awaiting approval (Solovic *et al.*, 2008).
Two-step model (TST positive followed by IGRA) is indicated in:
- Before starting anti-TNF-alpha therapy: regularly, once a year, for patients subjected to long-term anti-TNF therapy.
- Healthcare workers exposed to open forms of TB.
- Contacts.
- Military personnel after serving in TB endemic countries.
- As a part of medical examination of risk groups in the population-refugees, minorities.
- As a part of differential diagnosis of pulmonary and extrapulmonary TB.

The KNCV/EuroTB statement
The use of IGRAs in low- and medium- prevalence countries in Europe is stated in a Consensus Statement of a Wolfheze Workshop organized by KNCV/EuroTB, Vilnius, September 2006 (KNCV/EuroTB. 2006). According to this statement, Interferon gamma release assays (IGRAs) offer an alternative to tuberculin skin testing for the diagnosis of LTBI or as an additional diagnostic method for active TB. Public health specialists involved in TB control, mainly in European countries with low and intermediate incidence of TB, met in Vilnius, Lithuania, in September 2006, to consider the use of IGRA as-

says because of the increasing demand for the use of these assays. There was consensus on the value of the use of IGRAs for the diagnosis of LTBI based on the following agreed points:

- Although there is no clear gold standard for LTBI, IGRAs, in published contact tracing incidents, reflect the degree of exposure to infectious cases more accurately than does TST. This suggests that IGRAs are more specific than TST. Discordant results between IGRAs and TST, however, cannot be completely explained by the notion that IGRAs are more specific with regard to cross-reaction with non-tuberculous mycobacterial (NTM) infections or with the Bacille Calmette Guerin (BCG) vaccine.
- Both commercial systems (QFT and TSPOT) probably perform well for LTBI detection in immunocompetent individuals.
- Studies of IGRA sensitivity suggest they are at least as sensitive as TST in TB patients but may be less sensitive than TST for detecting LTBI in immunocompetent individuals.
- Theoretically, a combination of TST (with its high sensitivity) followed by IGRAs (with their greatest specificity) should be an optimal approach for contact tracing in incidents where there is a known index or source case. Clearly this advantage is negated where the patient does not return for reading of the TST. In those cases, the single-visit IGRA would be more advantageous.
- Although it is reasonable to assume that a positive IGRA is as predictive of later active TB as a positive TST, there is no evidence so far to suggest a higher or lower degree of predictability.
- IGRAs are of value for diagnosing/excluding LTBI in children or HIV- positive (or other immuncompromised) individuals, including those about to receive anti-tumour necrosis factor or other immunomodulating therapy.

IGRAs are of value in any situation requiring serial TST testing, e.g. occupational health-related screening/exposure.

Summary of the present guidelines on IGRAs

In summary, with all the different suggestions used by the different countries for the correct use of the IGRA tests, it can be observed that:

- The two-step approach seems to be the most favored strategy for IGRA use.
- The two-step approach is particularly favored in contacts, especially BCG- vaccinated contacts.
- A trend towards using IGRAs alone prior to anti-TNF-alpha therapy.
- Some guidelines are still cautious about IGRA use in young children.
- Few guidelines recommend IGRAs for active TB, but some recommend it as an adjunct, especially in children.
- Most guidelines do not mention use for serial testing of HCWs.
- While some guidelines have been updated to keep up with the evidence base (e.g. USA, Canada, Germany), others have yet to be updated (e.g. UK, Australia, France, Czech).
- Few of the guidelines used evidence summaries and systematic reviews. Most are based on narrative literature reviews and expert opinion.
- Most guidelines did not include a clear description of potential conflicts of interests and industry involvement in guideline development.

Cost effectiveness of IGRAs vs PPD skin test

There are several major IGRA clinical advantages that provide economic benefits for TB infection control programs. This can be summarised in:

- Single visit.
- High sensitivity (up to 93%) and specificity (>99%) for detecting active TB (Harada N *et al.,* 2008). *Avoids false positives due to BCG vaccination* (Mori *et al.,* 2004) *and most environmental non-tuberculous mycobacteria* (Anderson *et al.,* 2000).
- Unlike the TST it is not subject to errors in test placement or reading.
- Reduction in personnel cost - which is the major cost component of a TST program. *TST reagents represent less than 1.5% of the total cost of TST screening program* (Lambert *et al.,* 2003).
- Reduction in additional costs - such as chest X-rays - associated with investigating false positive cases (Nienhaus *et al.,*2008).
- Avoids boosting (Leyten *et al.,*2007). *Eliminates the need for two-step testing.* In situations with serial testing for *M. tuberculosis* infection, initial two-step testing - which is necessary with the TST - is unnecessary with the QuantiFERON-TB Gold and is not recommended" (CDC. 2005*).*

The use of both IGRA tests is a cost effective solution for TB screening when compared to the

significant costs associated with the tuberculin skin test (TST). Total costs for the tuberculin skin test are often believed to be lower than the actual cost. According to a published study by Lambert *et al.*, the total cost of a tuberculin skin test per healthcare worker tested ranged from $41.00USD to $362.00USD (Lambert *et al.,* 2003). In fact the source of many of the costs for the tuberculin skin test include:

1. Procedural:
   a) Two visits are required, one for administration of the PPD and the second for interpretation of the test result.
   b) Subjectivity of test administration and interpretation of results.
2. Operational:
   a) Training and retraining in proper test inoculation and interpretation.
   b) Getting the individual to return for the second visit.
   c) Following up on those who do not return for the second visit.
   d) Two-step testing for new employees requires a total of four visits.
3. Performance:
   a) The TST cross-reacts with the BCG vaccine resulting in false-positive results.
   b) TST results are affected by immunosuppression resulting in false-negative results (Lee *et al.,* 2006).
   c) The IGRA tests address the sources of these costs associated with the TST, Single blood test, only one visit required. IGRAs results are not affected by immunosuppression or BCG vaccine.

A number of economic assessments in using IGRAs respect to TST have been conducted so far in different countries. These are summarised here below.

Japan economic assessments

A cost-effectiveness analysis of QFT in a TB contact investigation in Japan was performed by Mori, Harada, 2005. A model was built assuming that a group of young people was exposed to an infection source with different degrees of intensity. The strategies for investigating this group included using QFT to test subjects with erythema size exceeding 30 mm, 20 mm and 10 mm, as compared with the strategy of only using TST or QFT. The analysis confirmed that the additional use of QFT would greatly reduce the number of indications for chemoprophylaxis of uninfected cases and that the use of QFT is cost effective.

UK economic assesments

UK National Institute for Health and Clinical Excellence (NICE). Tuberculosis clinical guideline - full guideline, second consultation (NICE, http,//www.nice.org.uk/guidance/index).

NICE considered the cost-effectiveness of 4 different strategies - no test, TST alone, IGRA alone and IGRA testing for individuals with a positive TST - for diagnosing LTBI. The assessment showed that:

- At all prevalence levels, an IGRA-only strategy was cheaper than a TST-only strategy.
- Overall, the two-stage TST/IGRA strategy was most cost effective, however the impact of false negative results or logistical issues involved with two-step testing was not considered in the assessment.
- One step IGRA testing can be used in individuals "in whom tuberculin skin testing may not be reliable" such as those with immune suppressing diseases (including HIV) or on immune suppressive treatment (e.g. corticosteroids), Hodgkin's disease, infectious mononucleosis and viral infections in general (including those of the upper respiratory tract).
- The NICE assessment showed that above a prevalence level of 40%, one-step testing (that is a single IGRA test) is the most cost effective option.

NICE guidelines state that "interferon-gamma tests showed little evidence of being affected by prior BCG vaccination, and showed stronger correlation with exposure categories than did TST. The specificity of interferon-gamma tests seemed better, and there was less potential for false positive results".

German economic assessments

A Markov model was used to assess the health and economic outcomes of isoniazid treatment of 20 year old TB contacts using two different TST cut-offs (5 mm and 10 mm), QFT alone and QFT as a confirmatory test for TST results (Diel *et al.,* 2007).

The number treated to prevent one TB case was 22 for the two QFT based procedures, 40 for the TST at a cut-off of 10 mm, and 96 for the TST at

a cut-off of 5 mm "which may appear to be a striking argument from an ethical point of view" for using only QFT.

This analysis showed that the two TST-based strategies "when performed alone, in each case [was] more costly and less effective than the Quantiferon®-TB Gold in-Tube assay, the higher cost of implementation of which was outweighed by the averted cost of unnecessarily treating contacts who otherwise would have been wrongly classified as LTBI cases".

Of the four strategies, QFT following the TST screening of close-contacts at a cut-off of 5 mm was the most cost-effective option, followed by the QFT alone strategy. However the cost of combining the two tests was "only marginally lower than the total cost of the program based on QFT-G assay alone per 1000 close-contacts by approximately $ 1,397 (0.61%)".

In another German cost-minimisation analysis (Diel *et al.,* 2006), the costs of investigating a cohort of adult tuberculosis (TB) contacts over a period of 2 years was calculated. In this assessment the total cost of simply administering the TST was € 19.24 per person (includes tuberculin material costs, as well as TST administration and reading costs) while the total cost of performing QFT was € 47.68 per person (includes blood sampling, sample transport and all laboratory material and labour costs). These costs DO NOT include follow up costs for those testing positive by either test. Such follow-up comprised three chest X-rays at a cost of € 74.3 per X-ray - which includes all labour and material costs of performing a chest X-ray. This analysis showed that: when TST was used alone the average costs for every contact followed amounted to € 91.

If instead of TST, QFT alone was performed, the cost per contact was reduced by 33% to € 61.

If both tests were combined (validation of a positive TST by QFT) the costs were reduced by 43% to € 52.

A two-step approach proved to be marginally cost effective compared to only using QFT. However this sacrifices the operational ease of only using QFT, but also has the risk of missing individuals (eg. Those with immunosuppression) with false negative TST results. As a result the authors state that "the TST/QFT-G two-step strategy should be reassessed in the presence of such specific epidemiological conditions".

USA/Canada economic assessments

In this assessment (De Perio *et al.,* 2007) a Markov model was used to compare the cost effectiveness of 3 strategies (QuantiFERON-TB Gold In-Tube, QuantiFERON-TB Gold and Tuberculin Skin Test (TST) for detecting LTBI in new health care workers (HCW) with or without prior BCG vaccination. It showed that for non-BCG vaccinated HCW, the incremental cost effectiveness of QuantiFERON-TB Gold, compared with QuantiFERONTB Gold In-Tube was $ US 14,092/QALY. For BCG vaccinated HCWs, the incremental cost-effectiveness of QuantiFERON-TB Gold was $US 103,020/QALY. Sensitivity analyses show that if the sensitivity of QuantiFERON-TB Gold In-Tube exceeds that of QuantiFERON-TB Gold, QuantiFERON-TB Gold In-Tube is the most effective and least costly strategy.

A cost effectiveness analysis was done by Oxlade O, Schwartzman K and Menzies D (Oxlade *et al.*, 2007). The researchers used a Markov model to compare the expected TB cases and costs of various screening methods among immigrants to Canada and TB contacts over a period of 20 years. Sequential screening with TST then QFT was more cost-effective than QFT alone in all scenarios and more cost-effective than TST alone in selected subgroups. In both immigrants and TB contacts who had received BCG vaccination after infancy, QFT was more cost-effective than TST, because of reduced TST specificity.

M.A. de Perio *et al.* (De Perio *et al.,* 2009) evaluated the cost-effectiveness of Interferon Gamma Release Assays vs. Tuberculin Skin Tests in Health Care Workers. The authors state that the QFT is more effective and less costly than the TST for detection of LTBI in both low- and high-prevalence populations, independent of BCG vaccination status.

At the 2nd Global Symposium on IGRAs in Dubrovnik, 2009, L. Masae Kawamura (Masae *et al.*, 2009) presented the Cost Effectiveness Analysis made in the San Francisco TB Control Section of the Department of Public Health at the Francis J. Curry National TB Center. It was found that the QFT-Gold in tube with investment in automation is the least costly IGRA (If IGRA chosen, investment in automation is easily economically justified). A model predicted IGRA is most cost-effective choice over wide range of assumptions.

The testing strategy for different populations should be selected based on local characteristics and experience.

Akiko Kowada from the Department of Health Service, Katsushika City Public Health Center in Tokyo, presented two studies at the 2nd Global Symposium on IGRAs in Dubrovnik, 2009, Cost effectiveness of IGRAs for tuberculosis contact screening and Cost effectiveness of IGRAs for annual tuberculosis healthcare worker screening (Kowada *et al.,* 2009). The main conclusions of the first study were:

1. The QFT alone strategy is the most cost effective for TB contact screening in Japan.
2. When the TST specificity is over 0.72, the TST followed by the QFT strategy is more cost effective than the QFT alone strategy at the level of $US 25,000/QALY gained as a willingness to pay.
3. The QFT alone strategy would be more cost effective in individuals at high risk of tuberculosis mortality, such as the elderly.

About the cost effectiveness of IGRAs for annual tuberculosis healthcare worker screening, it was stated that:

1. The QFT alone strategy is the most cost effective in BCG vaccinated healthcare workers in Japan.
2. When the probability of having LTBIs over 0.463, the QFT/Chest X ray strategy is more cost effective than the QFT alone strategy at the threshold of $US25,000/QALY as a willingness to pay.

CONCLUSIONS

IGRAs were successfully applied in both active and LTBI, pulmonary and extrapulmonary TB, patients with MDR TB, in immunocompromised individuals with HIV/AIDS. IGRAs could be successfully used in patients with transplantation, immune suppressive therapies, anti-TNF-alpha treatment, cancers, chronic renal failure and diabetes. In addition, the use of IGRAs is successful in children of any age, epidemiological studies (contact tracing, testing of HCWs), monitoring of anti-TB therapy and discrimination between BCG vaccination and MTB infection.

In immunocompromised hosts, all available data should be used to demonstrate or exclude latent infection with *MTB*. The risk of developing active TB differs among various immunocompromising conditions. However, screening for latent infection with *MTB* in immunocompromised patients is carried out irrespective of the type of immunosuppression, because the risk of developing active TB is probably higher than that of immunocompetent individuals. Sensitivity of the TST is limited in immunocompromised individuals and specificity is limited because of cross reactivity due to prior infection with environmental mycobacteria or BCG vaccination. IGRAs have a higher specificity in populations with a high prevalence of BCG vaccination compared with TST.

TB-contacts potentially benefiting from preventive therapy should be identified hierarchically according to likelihood of having become infected by a putative source and by presence of potentially aggravating risk factors. IGRAs may be superior to the TST in identifying contacts at risk of developing TB.

Children are more likely to develop TB than adults after exposure to an active TB case, hence contact screening and chemoprophylaxis are particularly important.

In children with a high risk of infection (especially young children aged <5 years and immunocompromised children) an IGRA should be performed in addition to the TST to increase sensitivity. If either test gives a positive result, this may be interpreted as supportive evidence of infection, and the children should be offered preventive chemotherapy.

Children aged 5 yrs with exposure to sputum smear-positive TB should also be screened and a positive TST be confirmed by IGRA, where available. In cases in which the treating pediatrician opts not to provide preventive therapy to TST positive but IGRA - negative children, surveillance for a minimum of 12-24 months is indicated for observation and to collect outcome data, until the positive and negative predictive value of IGRA are better established in the setting of paediatric TB.

In summary, the following approach could be recommended in accordance to the setting that should be tested:

- The two-step approach seems to be the most favored strategy for IGRA use.
- The two-step approach for use of IGRAs in young children.

- The two-step approach for active TB in children.
- The two-step approach for use of IGRAs in contacts, especially BCG- vaccinated.
- The use of IGRAs alone in HIV/AIDS patients.
- The use of IGRAs alone prior to anti-TNF-alpha therapy.
- The use of IGRAs alone in hemodialysis patients.
- The use of IGRAs alone in patients receiving immunosuppressive drug therapy.
- The use of IGRAs alone in patients with organ transplantation.
- The two-step approach for use of IGRAs in active TB.
- The two-step approach for use of IGRAs for serial testing of HCWs.
- The use of IGRAs' positive predictive value for the development of active TB is likely to be equal to or better than that of the TST for immunocompetent individuals.

REFERENCES

American Academy of Pediatrics. Report of the Committee on Infectios Diseases. AAP Red Book 2009, 28th Edition.

Anderson P., Munk ME., Pollock JM., Doherty TM. (2000). Specific immune-based diagnosis of TB. *Lancet.* **356**, 1099-1104.

Andersen P., Munk ME., Pollock JM., Doherty TM. (2000). Specific immune-based diagnosis of tuberculosis. *Lancet.* **356**, 1099-1104.

Arend S.M., Andersen P., van Meijgaarden K.E., Skjot R.L., Subronto Y.W., van Dissel J.T., Ottenhoff T.H. (2000). Detection of active tuberculosis infection by T cell responses to early-secreted antigenic target 6-kDa protein and culture filtrate protein 10. *J. Infect. Dis.* **181**, 1850-1854.

Australian Government., Department of Health and Ageing. National Tuberculosis Advisory Committee, Position statement on Interfero-gamma release immunoassays in the detection of latent tuberculosis infection, October 2007.

Australian Society for Infectious Diseases Refugee Health Guidelines Working Group. Diagnosis, management and prevention of infections in recently arrived refugees, 2009.

Behr M.A., Wilson M.A., Gill W.P., et al. (1999). Comparative genomics of BCG vaccines by whole genome DNA microarray. *Science.* **284**, 1520-1523.

Beglinger C., Dudler J., Mottet C., Nicod L., Seibold F., Villiger P., Zellweger J. (2007). Screening for tuberculosis infection before the initiation of an anti-TNF-alpha therapy. *Swiss Med. Wkly.* 620-622.

Brock I., Munk ME., Kok-Jensen A., Andersen P. (2001). Performance of whole blood IFN-gamma test for tuberculosis diagnosis based on PPD or the specific antigens ESAT-6 and CFP-10. *Int. J. Tuberc. Lung. Dis.* **5**, 462-467.

Canadian Tuberculosis Committee. (2008). Updated Recommendations on Interferon Gamma Release Assays for Latent Tuberculosis Infection. An Advisory Committee Statement (ACS). *Can. Commun. Dis. Rep.* **34** (ACS-6), 1-13.

Centers for Disease Control and Prevention (CDC). Emergence of Mycobacterium tuberculosis with extensive resistance to second-line drugs-worldwide., 2000-2004. (2006). *MMWR Morb. Mortal. Wkly. Rep.* **55**, 301-305.

CDC. Guidelines for Using the QuantiFERON®-TB Test for Diagnosing Latent Mycobacterium tuberculosis Infection. MMWR., January 31, 2003/52 (RR02). 15-18.

CDC/NIH/IDSA Guidelines 2009. Guidelines for prevention and treatment of Opportunistic infections in HIV-infected adults and adolescents. MMWR (2009) 58.

Centers for Disease Control. MMWR Dec 16 (2005). Vol. **54**, No. RR-15.

Committee of Prevention, Japanese Society of Tuberculosis. (2006). Kekkaku. **81**, 393-397.

Commissie voor Praktische Tuberculosebestrijding (CPT). Plaatsbepaling van de Interferon Gamma Release Assays bij de diagnostiek van tuberculose. [Dutch Committee for Practical Tuberculosis Control. Positioning of Interferon Gamma Release Assays in the diagnosis of tuberculosis]. October 2007

Diel R., Forßbohm M., Loytved G., Haas W., Hauer B., Maffei D., Magdorf K., Nienhaus A., Rieder HL., Schaberg T., Zellweger JP., Loddenkemper R. (2007). German Central Committee Against Tuberculosis (Deutsches Zentralkomitee zur Bekämpfung der Tuberkulose - DZK). [Recommendations for contact tracing in tuberculosis (Empfehlungen für die Umgebungsuntersuchungen bei Tuberkulose]. *Pneumologie.* **61**. 440.

Diel R., Hauer B., Loddenkemper R., Manger B., Krüger K. (2009). New TB testing recommendations for autoimmune diseases., Germany. (Recommendations for tuberculosis screening before initiation of TNF-α-inhibitor treatment in rheumatic diseases. Zeitschrift für Rheumatologie., Volume 68, Number 5, July.

Diel R., Nienhaus A., Loddenkemper R. (2007). Cost-effectiveness of interferon gamma release assay screening for latent tuberculosis infection treatment in Germany. *Chest.* **5**, 1424-1434.

Diel R., Nienhaus A., Lange C., Schaberg T. (2006). Cost optimization of screening for latent tubercu-

losis in close contacts. *Eur. Respir. J.* **28**, 35-44.

De Perio MA., Tsevat J., Roselle GA., Kralovic SM., Eckman MH. (2007). The cost-effectiveness of Interferon-γ release assays vs. Tuberculin skin tests for detecting latent tuberculosis infection in healthcare workers. Presented at Infectious Diseases Society of America. Oct.

De Perio M.A., Tsevat J., Roselle G.A., Kralovic S.M., Eckman M.H. (2009). Cost-effectiveness of interferon gamma release assays vs tuberculin skin tests in health care workers. *Arch. Intern. Med.* **169**, 179-187.

Elaborato dei Gruppi di Studio Infezioni e Tubercolosi dell'Associazione Italiana Pneumologi Ospedalieri (AIPO) e della Società Italiana di Medicina Respiratoria (SIMeR). (2006). Documento sull'utilizzo dei nuovi test immunologici per la diagnosi di infezione tubercolare latente.

Girardi E. (2009). Italian National Guidelines for TB Control. [Draft].

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

Gomez J.E., McKinney J.D. (2004). M. tuberculosis persistence, latency, and drug tolerance. *Tuberculosis* (Edinb). **84**, 29-44.

Harada N., Higuchi K., Yoshiyama T., Kawabe Y., Fujita A., Sasaki Y., Horiba M., Mitarai S., Yonemaru M., Ogata H., Ariga H., Kurashima A., Wada A., Takamori M., Yamagishi F., Suzuki K., Mori T., Ishikawa N. (2008). Comparison of the sensitivity and specificity of two whole blood interferon-gamma assays for M. tuberculosis infection. *J. Infect.* **56**, 348-353.

Haute Authorité de Santé. (2006). Test de détection de la production d'Inteferféron g pour le diagnostic des infections tuberculeuses.

Health Protection Agency. (2007). Position Statement on the use of Interferon Gamma Assay (IGRA) test for Tuberculosis (TB). October. Available online at www.hpa.org.uk.

Ulrichs T., Munk M.E., Mollenkopf H., Behr-Perst S., Colangeli R., Gennaro M.L., Kaufmann S.H. (1998). Differential T cell responses to Mycobacterium tuberculosis ESAT6 in tuberculosis patients and healthy donors. *Eur. J. Immunol.* **28**, 3949-3958.

Huebner R.E., Schein M.F., Bass J.B. Jr. (1993). The tuberculin skin test. *Clin. Infect. Dis.* **17**, 968-975.

Iademarco M.F., Castro K.G. (2003). Epidemiology of tuberculosis. *Seminars in respiratory infections* **18**, 225-240.

Jensen P.A., Lambert L.A., Iademarco M.F., Ridzon R., Dheda K. (2005). Guidelines for preventing the transmission of Mycobacterium tuberculosis in health care settings 2005. MMWR *Recomm. Rep.* **54**, 1-141.

Kowada A. (2009). Cost effectiveness of IGRAs in Japan. 2nd Global Symposium on IGRAs., Dubrovnik May 30-June 1.

KNCV/EuroTB Use of Gamma-interferon assays in low- and medium-prevalence countries in Europe, a consensus statement of a Wolfheze Workshop organised by KNCV/EuroTB., Vilnius. Sept 2006.

Lalvani A., Pathan A.A., McShane H., et al. (2001). Rapid detection of Mycobacterium tuberculosis infection by enumeration of antigen-specific T cells. *Am. J. Respir. Crit. Care Med.* **163**, 824-828.

Lambert L., Rajbhandary S., Quails N, Budnick L., Catanzaro A., Cook S., Daniels-Cuevas L., Garber E., Reves R. (2003). Costs of implementing and maintaining a tuberculin skin test program in hospitals and health departments. *Infect. Control Hosp. Epidemiol.* **24**, 814-820.

Lambert L., et al. (2003). Costs of Implementing and Maintaing a Tuberculin Skin Test Program in Hospitals and Health Departments. *Inf. Con. and Hosp. Epidm.* 814-821

Lee J., et al. (2006). Comparison of two commercial interferon-gamma assays for diagnosing Mycobacterium tuberculosis infection. *Eur. Respir. J.* **28**, 24-30.

Leyten E.M., et al. (2007). Effect of tuberculin skin testing on a Mycobacterium tuberculosis-specific IFN-γ assay. *Eur. Respir. J.* **29**, 1212-1216.

Mahairas G.G., Sabo P.J., Hickey M.J., Singh D.C., Stover C.K. (1996). Molecular analysis of genetic differences between Mycobacterium bovis BCG and virulent M. bovis. *J. Bacteriol.* **178**, 1274-1282.

Masae Kawamura L. (2009). Tuberculosis screening of contacts and HCWs in San Francisco. 2nd Global Symposium on IGRAs., Dubrovnik May 30-June 1.

Mazurek G.H., Jereb J., Lobue P., Iademarco M.F., Metchock B., Vernon A. (2005). Guidelines for using the QuantiFERON-TB Gold test for detecting Mycobacterium tuberculosis infection, United States. *MMWR Recomm. Rep.* **54**, 49-55.

Mori T., Sakatani M., Yamagishi F., Takashima T., Kawabe Y., Nagao K., Shigeto E., Harada N., Mitarai S., Okada M., Suzuki K., Inoue Y., Tsuyuguchi K., Sasaki Y., Mazurek G.H., Tsuyuguchi I. (2004). Specific detection of tuberculosis infection, an interferon-gamma-based assay using new antigens. *Am. J. Respir. Crit. Care Med*. **170**, 59-64.

Mori T., Harada N. (2005). Cost-effectiveness analysis of QuantiFERON-TB 2nd generation used for detection of tuberculosis infection in contact investigations. *Kekkaku.* **80**, 675-686.

National Immunisation Advisory Committee of the Royal College of Physicians of Ireland. Immunisation Guidelines for Ireland., 2008 Edition.

Nienhaus A., Schablon A., Le B cle C., Siano B., Diel R. (2008). Evaluation of the interferon-release assay

in healthcare workers. *Int. Arch. Occup. Environ. Health.* **81**, 295-300.

OXLADE O., SCHWARTZMAN K., MENZIES D. (2007). Interferon-gamma release assays and TB screening in high-income countries, a cost/effectiveness analysis. *Int. J. Tuberc. Lung. Dis.* **11**, 16-26.

PAI M. (2009). Guidelines on IGRA, corcondant or discordant? 2nd Global Symposium on IGRA. Dubrovnik, May 30 - June 1.

PANEL ON ANTIRETROVIRAL GUIDELINES FOR ADULT AND ADOLESCENTS. (2008). Guidelines for the use of antiretroviral agents in HIV-1-infected adults and adolescents. US Department of Health and Human Services. Jan 29. 1-128.

RAVN P., DEMISSIE A., EGUALE T., WONDWOSSON H., LEIN D., AMOUDY H.A., MUSTAFA A.S., JENSEN A.K., HOLM A., ROSENKRANDS I., OFTUNG F., OLOBO J., VON REYN F., ANDERSEN P. (1999). Human T cell responses to the ESAT-6 antigen from Mycobacterium tuberculosis. *J. Infect. Dis.* **179**, 637-645.

RECOMMENDATIONS OF THE SPANISH SOCIETY OF PULMONOLOGY AND THORACIC SURGERY (SEPAR). (2008). Diagnosis and Treatment of Tuberculosis. Arch Bronconeumol. **44**, 551-566.

SOLOVIC I. (2008). National Institute for TB., Lung Diseases and Thoracic Surgery, Slovakia.

SORENSEN A.L., NAGAI S., HOUEN G., ANDERSEN P., ANDERSEN A.B. (1995). Purification and characterization of a low-molecular-mass T-cell antigen secreted by Mycobacterium tuberculosis. *Infect. Immun.* **631**, 710-717.

SWISS LUNG LEAGUE. (2005). Erkennung der Tuberkuloseinfektion mittels Bluttest (Interferon-gamma). Detection of latent tuberculosis using a blood test (Interferon-Gamma). Berne, Swiss Federal Office of Public Health (actualised 19 December 2005).

THWAITES G., FISHER M., HEMINGWAY C., SCOTT G., SOLOMON T., INNES J. (2009). British Infection Society guidelines for the diagnosis and treatment of tuberculosis of the central nervous system in adults and children. *J. Infect.* **59**, 167-87.

TUFARIELLO J.M., CHAN J., FLYNN J.L. (2003). Latent tuberculosis, mechanisms of host and bacillus that contribute to persistent infection. *Lancet Infect. Dis.* **3**, 578-590.

UK NATIONAL INSTITUTE FOR CLINICAL EXCELLENCE (NICE). (2006). National clinical guideline for diagnosis, management, prevention and control of tuberculosis and measures for its prevention and control. London, Royal College of Physicians.

UK NATIONAL INSTITUTE FOR HEALTH AND CLINICAL EXCELLENCE (NICE). Tuberculosis clinical guideline - full guideline, second consultation. http,//www.nice.org.uk/guidance/index.

WORLD HEALTH ORGANIZATION (WHO). (2006). Tuberculosis Fact sheet N° 104 - Global and regional incidence. March 2006. Retrieved on 6 October.

VAN PINXTEREN L.A., RAVN P., AGGER E.M., POLLOCK J., ANDERSEN P. (2000). Diagnosis of tuberculosis based on the two specific antigens ESAT-6 and CFP10. *Clin. Diagn. Lab. Immunol.* **7**, 155-160.