Immunochromatographic IgG/IgM Test for Rapid Diagnosis of Active Tuberculosis[▽]

Walid Ben-Selma,* Hedi Harizi, and Jalel Boukadida

Microbiology and Immunology Laboratory, UR02SP13, CHU Farhat Hached, Sousse, Tunisia

For rapid diagnosis and discrimination between active tuberculosis (TB) and other pulmonary diseases, we evaluated the clinical usefulness of detection of serum immunoglobulin IgG and IgM antibodies raised against mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens by a commercial rapid immunochromatographic IgG/IgM test (Standard Diagnostics, South Korea) in 246 serum samples from three groups of patients: (i) 171 patients with active TB (128 with pulmonary TB [pTB] and 43 with extrapulmonary TB [epTB]), (ii) 73 patients with pulmonary non-TB diseases, and (iii) two leprosy patients. The sensitivities of IgG and IgM in patients with active TB (pTB and epTB) were 68.4% and 2.3%, respectively. IgG had the best performance characteristics, with sensitivities of 78.1% and 39.5% in sera from patients with active pTB and epTB, respectively, and a specificity of 100%. The sensitivities of IgM were poor and were similar for pTB and epTB (2.3%). In contrast, specificity was very elevated (100%). The combination of IgG with IgM did not improve its sensitivity. IgG-mediated responses against the mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens might constitute a clinically useful tool for presumptive diagnosis and discrimination of active pTB from other pulmonary diseases. Moreover, based on its simplicity and rapidity of application, it could be a screening tool for active pTB in poorly equipped laboratories.

Tuberculosis (TB) is still a serious public health problem in the world, with about 8.9 to 9.9 million new cases and 1.3 million deaths occurring worldwide annually (45); it has been estimated that one-third of the world's population is infected with *Mycobacterium tuberculosis* (*MT*) (41). One of the principles of TB control is rapid and accurate diagnosis of infected patients in order to allow prompt initiation of antibiotic therapy and to prevent transmission (2). Microscopic examination of sputum is a rapid, technically simple, and inexpensive test available for the routine diagnosis of TB in most developing countries. However, sputum smear microscopy with Ziehl-Neelsen staining is only 60 to 70% sensitive for the diagnosis of pulmonary TB (pTB) compared with the sensitivity of sputum culture (27, 29). Mycobacterial cultures are more sensitive but take at least 2 weeks, or longer if solid media are used, and culture facilities are not available in many countries. On the other hand, the lack of radiological appearances specific to TB makes chest radiography a relatively subjective and error-prone practice for the diagnosis of pTB (28). In addition, coinfection with HIV may change the clinical presentation of TB and reduce the sensitivity of classical microbiology methods (12, 20, 31). Therefore, in developing countries, it is especially important to have an inexpensive and rapid test for TB identification so that infected individuals can be isolated and treated immediately (21).

There is an urgent need for rapid, cost-effective, and accurate methods for the diagnosis of active TB. A serological test may be attractive because it would be relatively rapid and would not require sputum expectoration. Challenges for the development of effective serological tests include the need to discriminate active TB disease from other pulmonary non-TB diseases, such as bronchitis, cancer, and pneumonia, that can mimic both the clinical and the radiographic symptoms of pTB patients (35) and the need for consistent performance with genetically and immunologically diverse populations.

Several purified mycobacterial antigens, such as 85A antigen, lipoarabinomannan, plasma membrane antigen, and antigen 60 (A60), have been used for the serodiagnosis of TB, as we and other have previously reported (5, 6, 16, 22, 47). The 38-kDa protein is an immunodominant lipoprotein antigen isolated as a component of antigen 5 by affinity chromatography and is specific only for the *M. tuberculosis* complex (11, 39, 43). It is one of the most important immunogenic antigens of *M. tuberculosis* (23, 25), inducing B- and T-cell responses with high specificity for TB, and is considered a prime candidate for the development of new diagnostic reagents for diagnosis of active TB (8). The 38-kDa antigen is also a core component in various commercial serological tests (Pathozyme TB complex kit [Omega Diagnostics, Alloa, Scotland], Pathozyme Myco kits for IgG, IgM, and IgA [Omega Diagnostics], Rapid TB test [Quorum Diagnostics, Vancouver British, Columbia, Canada], and ICT Tuberculosis AMRAD-ICT [Amrad, Sydney, Australia]). Antibodies to the 38-kDa antigen occur in a high percentage of TB patients and provide the serodiagnostic test with the most favorable characteristics described to date (23, 36, 43).

The 16-kDa antigen is an immunodominant antigen, frequently called 14-kDa antigen, related to the family of low-molecular-weight heat shock proteins (HSP). This antigen contains B-cell epitopes specific for the *M. tuberculosis* complex (19, 33). Furthermore, it has been suggested that the 16-kDa antigen is immunogenic in the early stages of infection with *M.*

* Corresponding author. Mailing address: Microbiology and Immunology Laboratory, UR02SP13, CHU Farhat Hached, Av. Ibn el Jazzar 4000, Sousse, Tunisia. Phone and fax: 216 73219504. E-mail: wbenselma@hotmail.com.

tuberculosis and in primary TB (9). This antigen has shown considerable promise as a serodiagnostic target in assay protocols based on monoclonal antibody (MAb) competition and direct enzyme-linked immunosorbent assay (ELISA) formats (10, 26).

The 6-kDa antigen, characterized and purified in 1995 by Sorensen et al. (40), was subsequently used in different studies as an antigen with diagnostic potential, since it was found to be specific for *M. tuberculosis* and absent in *Mycobacterium bovis* BCG and in most environmental mycobacteria. The 6-kDa antigen stimulates T cells from patients with active TB, leading to an increase in gamma interferon production (4).

Many serological methods for the diagnosis of active TB have been designed and commercialized. Recently, we have evaluated commercial ELISAs for detection of antibody responses raised against mycobacterial antigens such the 38-kDa antigen, the 16-kDa antigen, lipoarabinomannan (6), and A60 (5). In this study, we evaluated a rapid immunochromatographic test to detect antibody directed against any one of the three 38-kDa, 16-kDa, and 6-kDa recombinant antigens in TB patients in Sousse, Tunisia, a region characterized by a moderate TB prevalence (9.5 new cases per 100,000 population) and incidence (21 cases/100,000/year) and a predominant *M. tuberculosis* strain (46).

MATERIALS AND METHODS

Setting. The present study was conducted from July 2007 to December 2010 in the Laboratory of Microbiology and Immunology, Farhat Hached University Hospital, Sousse, Tunisia, a setting with a moderate incidence (21 cases/100,000/ year) of TB (46) and a predominant *M. tuberculosis* strain. Informed written consent was obtained from all individuals prior to blood sampling, and this study was approved by the ethics committee of the Farhat Hached University Hospital.

A total of 246 total serum samples were obtained: 171 from patients with active TB, 73 from patients without evidence of TB, and 2 from subjects with leprosy. All subjects had previously been BCG vaccinated. None of the individuals, including patients with active TB and controls, had a history of severe pathologies, including HIV infection and cardiovascular disease. Demographic data, including sex, age, and clinical types of TB, are summarized in Table 1. Serum specimens were obtained upon admission before any therapy and stored at −80°C until immunochromatographic testing.

Study population. Serum specimens were obtained from the following three groups.

(i) Active tuberculosis group. Inclusion criteria for patients in the active tuberculosis group were determined according to the recommendation defined by the American Thoracic Society (2). The diagnostic criterion for the presence of TB disease was defined as the presence of at least one of the following: (i) clinical and radiological findings consistent with TB disease and positive sputum smears for acid-fast bacilli; (ii) culture positivity of sputum, bronchial lavage, or pleural fluid, as well as samples from organ systems other than the lung, for *M. tuberculosis*; and (iii) pathological evidence of TB disease in biopsy materials by histological examination. The sera were obtained from 171 patients in two distinct groups: 128 patients with a diagnosis of pulmonary TB and 43 patients with a diagnosis of an extrapulmonary form of TB (epTB) (Table 1).

(ii) Nontuberculous lung disease group. The control population consisted of 73 patients without clinical evidence of TB who were being treated for underlying disorders other than mycobacteriosis (Table 1).

(iii) Leprosy group. An additional control group was established with two cases of leprosy (Table 1). These cases were confirmed bacteriologically using smear microscopy.

Immunochromatographic test. The SD Bioline TB IgG/IgM immunochromatographic test is based on the detection of IgG and IgM raised against one or more of the three 38-kDa, 16-kDa, and 6-kDa recombinant mycobacterial antigens. The test device has letters C (control line), M (TB IgM test line), and G (TB IgG test line) on the surface of the case. The inner test strip is composed of (i) a conjugate pad containing recombinant TB-specific antigens (38 kDa, 16 kDa, and 6 kDa) conjugated with colloidal gold and (ii) a nitrocellulose membrane containing C (control), M (TB IgM test), and G (TB IgG test) lines. The

TABLE 1. Demographic and clinical data for TB patients and controls

Study group and infection site or condition[a]	No. of cases	No. male:no. female	Mean age, yr (range)
pTB	128	84:41	44 (14–78)
epTB			
Lymph nodes	27	8:17	43 (20–81)
Genitourinary tract	3	0:3	38 (30–42)
Meningitis	1	0:1	33
Mammary	1	0:1	24
Bones and joints	2	2:0	38 (24–52)
Pleuritis	9	7:2	30 (24–52)
Non-TB			
Asthma	38	50:25	60 (47–81)
COPD	16	10:6	63 (38–82)
Cancer	15	12:3	58 (24–73)
Aspergillosis	2	2:0	40 (30–50)
Mycosis	1	1:0	45
Sarcoidosis	1	1:0	37
Leprosy	2	2:0	62 (58–65)

[a] pTB, pulmonary tuberculosis; epTB, extrapulmonary tuberculosis; non-TB, pulmonary nontuberculosis diseases; COPD, chronic obstructive pulmonary disease.

M line is precoated with monoclonal anti-human IgM, the G line is precoated with monoclonal anti-human IgG, and the C line is precoated with goat anti-TB antibody. The control line is used for procedural control, and it should always appear if the test procedure is performed properly and the test reagents of the control line are working. During the test, TB IgG and/or TB IgM, if present in the specimen, migrates through the conjugate pad, where it binds to the conjugates. The antibody conjugates are then captured by anti-human IgM immobilized on the M line or the specific reagents to TB IgG immobilized on the G line of the membrane, forming a colored band on the test region (M and/or G), indicating a positive result.

Immunochromatographic testing was performed according to the manufacturer's instructions. Briefly, 10 µl of serum was added to the square sample well, and then 4 drops (90 to 120 µl) of assay diluents provided in the kit was added to the round diluent well. Interpretation of the test results was completed in 15 min after sample application. Each sample was tested in duplicate by two investigators without prior knowledge of the diagnosis status of each sample. Specimens were labeled with sequential numbers and processed blindly. This immunochromatographic test does not require special equipment and technical skill.

Statistical analysis. Sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) were calculated with Epi Info 6.0 software. All statistical tests were conducted using SPSS for Windows statistical software (version 15.0; SPSS, Chicago, IL). Differences were accepted as significant when the P value was <0.05.

RESULTS

In this study, we characterized the antibody response during active pTB and epTB by detecting IgG and IgM antibodies raised against the mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens in 171 patients with active TB (128 with pTB and 43 with epTB), 73 patients with pulmonary diseases other than TB, and 2 leprosy patients. The results obtained with the immunochromatographic test kit are shown in Tables 2 to 5.

IgG test. The results for detection of IgG against the mycobacterial 38-, 16-, and 6-kDa antigens are displayed in Table 2. The positivity rate of this test was around 68.4% in the active TB group (pTB plus epTB). When this group was subdivided into pTB and epTB subgroups, the positivity rate increased

TABLE 2. Sensitivities and specificities of IgG in active tuberculosis cases

Disease type[a]	No. (%) of positive cases	Sensitivity, % (95% CI)	Specificity, % (95% CI)	P
pTB + epTB	117 (68.4)	68.4 (60.8–75.2)	100 (93.9–100)	10^{-8}
pTB	100 (78.1)	78.1 (69.8–84.7)	100 (93.9–100)	10^{-8}
epTB	17 (39.5)	39.5 (25.4–55.6)	100 (93.9–100)	10^{-8}
Non-TB	0 (0)			

[a] Non-TB, nontuberculosis disease; epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.

considerably in the pTB group, to 78.1%, and decreased in the epTB group, to 39.5%. Thus, the sensitivities of the IgG test in the pTB and epTB groups were 78.1% (95% confidence interval [95% CI], 69.8 to 84.7%) and 39.5% (range, 25.4 to 55.6%), respectively.

For all subjects with pulmonary diseases other than TB, the assay provided negative results. Thus, the same high specificity and PPV (100%) were observed for both the pTB and epTB groups, and the NPVs were similar (Table 3).

IgM test. The IgM test detected a very low proportion (2.3%) of patients with active TB in the different groups (Table 4). Given the modest sensitivity, no cross-reaction was observed in the nontuberculosis group. Thus, high specificity and PPV (100%) were obtained (Table 5).

Combined use of IgG and IgM tests. When the results of both tests were considered, the number of positive results did not increase in patients with either pTB or epTB. Additionally, this combination did not improve the sensitivity of any test.

DISCUSSION

Rapid diagnostic tests for TB are required to facilitate early treatment of TB and prevention of *M. tuberculosis* transmission. Incorporation of multiple antigens in a diagnostic test may increase the sensitivity of an antibody-based test. This study evaluated the sensitivities and specificities of the multiantigen SD Bioline TB IgG/IgM test for the detection of active tuberculosis. We report, for the first time, that the IgG antibody response raised against a panel of the most important mycobacterial antigens, the 38-kDa, 16-kDa, and 6-kDa antigens (8, 9, 23, 36, 42), could be a significant marker for active pTB and could complement conventional bacteriological tests for the rapid diagnosis and discrimination of this clinical TB form from other pulmonary diseases having similar symptomatology. This test is rapid and easy to perform and could be a significant adjunct in diagnosis, particularly in resource-poor areas. This multiantigen immunochromatographic test is feasible in laboratories, with minimal technical requirements for testing.

The protective immune response in TB is cell mediated, but an antibody response is common and may be correlated with

TABLE 3. Positive and negative predictive values of IgG in active tuberculosis cases

Disease type[a]	PPV, % (95% CI)	NPV, % (95% CI)
pTB + epTB	100 (96–100)	58.1 (49.1–66.7)
pTB	100 (95.4–100)	72.8 (63–80.9)
epTB	100 (77.1–100)	74.3 (64.4–82.2)

[a] epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.

the lack of an effective cell-mediated response (7, 14, 17). Compared to assays of cell-mediated response, detection of antibodies is considerably simpler and less expensive (13), so serodiagnosis of TB has been extensively investigated (13, 15). However, after almost a century of effort, serological tests still have suboptimal sensitivity and specificity (15). The poor sensitivity may be due to substantial and unexplained heterogeneity of antibody responses to different antigens of *M. tuberculosis* among patients with active TB (32) and provides the rationale for development of multiantigen tests (1, 3). Although the poor specificity limits the utility of serological tests for diagnosis, the occurrence of false positives may provide insight into the immunopathogenesis of TB.

In this study, sensitivities of the IgG test were very high compared to those of the IgM test. These results were in agreement with previously reported data (18, 24, 43). Thus, several studies have been focused on the detection of IgG, IgM, and IgA against the 38-kDa antigen (18, 24, 43). IgA and IgM test sensitivities were significantly lower than that of IgG assays (18, 24, 43). Sensitivities ranged widely (from 16% to 94%) and specificities ranged slightly (from 93% to 100%), depending upon the smear status of TB patients and selection of patient populations in each study (18, 36, 38, 43). However, in our study, all pTB cases were smear microscopy and culture positive for *M. tuberculosis*. Smear microscopy status did not appear to be a determining factor for the heterogeneity of IgA, IgM, and IgG responses to the 38-kDa antigen in this study.

We suggest that the heterogeneity of the rates of positivity of immunochromatographic IgG and IgM antibodies to mycobacterial 38-kDa, 16-kDa, and 6-kDa antigens depends on the phase of TB. Thus, in primary pulmonary TB, IgM titers were initially high but IgG titers were very low, which results in low positivity of IgG. In postprimary pulmonary TB, low titers of IgM and high titers of IgG could explain high rates of positivity of IgG. In our study, the high positive rate for IgG could be due to one or both of the following factors. (i) It could be related to delays in consulting a pneumologist for diagnosis of a highly suspected active TB. According to previously published data (18), the sensitivity of the tests also depends on the phase of the disease and on the presence of mycobacteria in sputum. In chronic and culture-positive cases, antigenic stimulation persists, and if the patient is genetically able to mount an antibody response to the specific antigens, antibody levels are expected to be elevated (18). (ii) It could be related to prolonged exposure to *M. tuberculosis* in our setting, in which TB was moderately prevalent (46).

According to the recommendations of the World Health Organization (WHO) (44), to replace the "gold standard" culture test, a serological test should possess sensitivities of more than 80% and specificities of more than 95%. Our findings are

TABLE 4. Sensitivities and specificities of IgM in active tuberculosis cases

Disease type[a]	No. (%) of positive cases	Sensitivity, % (95% CI)	Specificity, % (95% CI)	P[b]
pTB + epTB	4 (2.3)	2.3 (0.8–6.3)	100 (93.9–100)	0.23*
pTB	3 (2.3)	2.3 (0.6–7.2)	100 (93.9–100)	0.24*
epTB	1 (2.3)	2.3 (0.1–13.8)	100 (93.9–100)	0.36*
Non-TB	0 (0)			

[a] Non-TB, nontuberculosis disease; epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.
[b] *, P value determined by Fisher's exact test (TB versus control).

in agreement with WHO recommendations for the clinical utility of the single use of this new immunochromatographic test because of its specificity and tendency (in term of sensitivity) for rapid diagnosis of active pTB.

In clinical practice, delayed diagnosis of active TB is an important problem in many general hospitals in industrialized countries because it results in greater patient morbidity and mortality and intrainstitutional spread of TB (30, 34, 37). Thus, it is very important to rapidly and correctly diagnose TB before the onset of antituberculosis therapy. In this setting, the elevated values of sensitivity, and especially positive predictive value, of detecting IgG raised against the 38-kDa, 16-kDa, and 6-kDa antigens in the pTB group compared to the group with non-TB pulmonary disease argue for the utility of clinical use of this test.

There were a number of limitations to the current study. (i) Serology testing was done retrospectively, using stored frozen sera. It is possible that the use of fresh serum may increase sensitivity. (ii) *M. tuberculosis* culture was used as the reference standard for the diagnosis of pTB. It is possible that some patients suspected of having pTB had culture-negative pTB disease and were improperly assigned to the group considered not to have pTB, thereby reducing the observed specificities of the test. However, this is unlikely since all cases included in our study are smear positive. Future studies of this test should include cases that are smear negative and culture positive. (iii) Patients from a single geographic region were included in this study, limiting the certainty that these data apply to other regions. (iv) The size of the studied populations should be expanded, and the question of whether this test is useful in the diagnostic of epTB remains. (v) The serological test has not been studied in cases where other pulmonary infections are implicated.

In summary, the main findings of the current study were as follows. (i) Clinical use of a multiple-antigen immunochromatographic test, specifically the SD Bioline TB IgG/IgM test, allowed detection of a significantly elevated number of patients with active pTB without a loss of specificity. (ii) This test can be used to facilitate and accelerate pTB diagnosis in smear-positive patients long before *M. tuberculosis* culture results are

available. (iii) The specificity of the test was not affected by BCG vaccination of adult patients. (iv) Its elevated sensitivity and NPV argue for the clinical usefulness of this test to discriminate between patients with active pTB and patients with non-TB diseases. (v) Serodiagnosis of active TB with a simple multiantigen immunochromatographic test is feasible in laboratories, with minimal technical requirements for testing. A better understanding of the dynamic repertoire of antibody responses in patients with *M. tuberculosis* infection and also other mycobacterial infections may facilitate the development of more sensitive and specific antibody-based methods for the diagnosis of active pTB disease. Further studies with large numbers of patients are required to confirm our results.

REFERENCES

1. **Al-Zahrani, K., et al.** 2000. Accuracy and utility of commercially available amplification and serologic tests for the diagnosis of minimal pulmonary tuberculosis. Am. J. Respir. Crit. Care Med. **162:**1323–1329.
2. **American Thoracic Society.** 2000. Diagnostic standards and classification of tuberculosis in adults and children. Am. J. Respir. Crit. Care Med. **161:**1376–1395.
3. **Amicosante, M., M. Houde, G. Guaraldi, and C. Saltini.** 1999. Sensitivity and specificity of a multi-antigen ELISA test for the serological diagnosis of tuberculosis. Int. J. Tuberc. Lung Dis. **3:**736–740.
4. **Arend, S. M., T. H. M. Ottenhoff, P. Andersen, and J. T. V. Dissel.** 2001. Uncommon presentation of tuberculosis: the potential value of a novel diagnostic assay based on the *Mycobacterium tuberculosis*-specific antigens ESAT-6 and CPF-10. Int. J. Tuberc. Lung Dis. **7:**680–686.
5. **Ben-Selma, W., et al.** 2010. Evaluation of the diagnostic value of measuring IgG, IgM, and IgA antibodies to mycobacterial A60 antigen in active tuberculosis. Diagn. Microbiol. Infect. Dis. **68:**55–59.
6. **Ben-Selma, W., et al.** 2010. Rapid detection of IgG against Mycobacterium tuberculosis antigens by two commercial ELISA kits. Int. J. Tuberc. Lung Dis. **14:**841–846.
7. **Bhatnagar, R., et al.** 1977. Spectrum of immune response abnormalities in different clinical forms of tuberculosis. Am. Rev. Respir. Dis. **115:**207–212.
8. **Bothamley, G. H., and R. M. Rudd.** 1994. Clinical evaluation of a serological assay using a monoclonal antibody (TB72) to the 38 kDa antigen of Mycobacterium tuberculosis. Eur. Respir. J. **7:**240–246.
9. **Bothamley, G. H., et al.** 1992. Specificity of antibodies and tuberculin response after occupational exposure to tuberculosis. J. Infect. Dis. **166:**182–186.
10. **Chandramuki, A., G. Bothamley, P. Brennan, and J. Ivanyi.** 1989. Levels of antibody to defined antigens of *Mycobacterium tuberculosis* in tuberculous meningitis. J. Clin. Microbiol. **27:**821–825.
11. **Chang, Z., A. Choudhary, R. Lathigra, and F. A. Quiocho.** 1994. The immunodominant 38-kDa lipoprotein antigen of Mycobacterium tuberculosis is a phosphate-binding protein. J. Biol. Chem. **269:**1956–1958.
12. **Colebunders, R., and I. Bastian.** 2000. A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int. J. Tuberc. Lung Dis. **4:**97–107.
13. **Daniel, T.** 1996. Immunodiagnosis of tuberculosis, p. 223–231. *In* W. M. Rom and S. Garay (ed.), Tuberculosis. Little, Brown & Co, Boston, MA.
14. **Daniel, T., M. Oxtoby, E. Pinto, and E. Moreno.** 1981. The immune spectrum in patients with pulmonary tuberculosis. Am. Rev. Respir. Dis. **123:**556–559.
15. **Daniel, T. M., and S. M. Debanne.** 1987. The serodiagnosis of tuberculosis and other mycobacterial diseases by enzyme-linked immunosorbent assay. Am. Rev. Respir. Dis. **135:**1137–1151.

TABLE 5. Positive and negative predictive values of IgM in active tuberculosis cases

Disease type[a]	PPV, % (95% CI)	NPV, % (95% CI)
pTB + epTB	100 (39.6–100)	31 (25.3–37.3)
pTB	100 (31–100)	37.5 (30.8–44.6)
epTB	100 (5.5–100)	64.1 (54.7–72.6)

[a] epTB, extrapulmonary tuberculosis; pTB, pulmonary tuberculosis.

16. **Daniel, T. M., et al.** 1986. Field evaluation of enzyme-linked immunosorbent assay for the serodiagnosis of tuberculosis. Am. Rev. Respir. Dis. **134:**662–665.

17. **David, H. L., et al.** 1992. Relationships between titer of antibodies immunoreacting against glycolipid antigens from *Mycobacterium leprae* and *M. tuberculosis*, the Mitsuda and Mantoux reactions, and bacteriologic loads: implications in the pathogenesis, epidemiology and serodiagnosis of leprosy and tuberculosis. Int. J. Lepr. Other Mycobact. Dis. **60:**208–224.

18. **Demkow, U., et al.** 2004. Antimycobacterial antibody level in pleural, pericardial and cerebrospinal fluid of patients with tuberculosis. Pneumonol. Alergol. Pol. **72:**105–110.

19. **Demkow, U., et al.** 2002. Humoral immune response against 38-kDa and 16-kDa mycobacterial antigens in bone and joint tuberculosis. Int. J. Tuberc. Lung Dis. **6:**1023–1028.

20. **Falloon, J.** 2000. Pulmonary manifestations of human inmunodeficiency virus infection, p. 1415–1426. *In* G. L. Mandell, J. E. Bennett, and R. Dolin (ed.), Mandell, Douglas, and Bennett's principles and practice of infectious diseases, 5th ed. Churchill Livingstone, New York, NY.

21. **Foulds, J., and R. O'Brien.** 1998. New tools for the diagnosis of tuberculosis: the perspective of developing countries. Int. J. Tuberc. Lung Dis. **2:**778–783.

22. **Gupta, S., S. Kumari, J. N. Banwalikar, and S. K. Gupta.** 1995. Diagnostic utility of the estimation of mycobacterial antigen A60 specific immunoglobulins IgM, IgA and IgG in the sera of cases of adult human tuberculosis. Tuber. Lung Dis. **76:**418–424.

23. **Harboe, M., and H. G. Wiker.** 1992. The 38-kDa protein of Mycobacterium tuberculosis: a review. J. Infect. Dis. **166:**874–884.

24. **Hendrickson, R. C., et al.** 2000. Mass spectrometric identification of Mtb 81, a novel serological marker for tuberculosis. J. Clin. Microbiol. **38:**2354–2361.

25. **Hoeppner, V., et al.** 1987. Appraisal of the monoclonal antibody based competition test for the serology of tuberculosis in Indonesia. Serodiagnosis Immunother. **1:**69–77.

26. **Jackett, P., et al.** 1988. Specificity of antibodies to immunodominant mycobacterial antigens in pulmonary tuberculosis. J. Clin. Microbiol. **26:**2313–2318.

27. **Kim, T. C., R. S. Blackman, K. M. Heatwole, T. Kim, and D. F. Rochester.** 1984. Acid-fast bacilli in sputum smears of patients with pulmonary tuberculosis. Prevalence and significance of negative smears pretreatment and positive smears post-treatment. Am. Rev. Respir. Dis. **129:**264–268.

28. **Koppaka, R., and N. Bock.** 2004. How reliable is chest radiography?, p. 51–60. *In* T. Frieden (ed.), Toman's tuberculosis case detection, treatment, and monitoring: questions and answers. World Health Organization, Geneva, Switzerland.

29. **Levy, H., et al.** 1989. A reevaluation of sputum microscopy and culture in the diagnosis of pulmonary tuberculosis. Chest **95:**1193–1197.

30. **Liam, C. K., and B. G. Tang.** 1997. Delay in the diagnosis and treatment of pulmonary tuberculosis in patients attending a university teaching hospital. Int. J. Tuberc. Lung Dis. **1:**326–332.

31. **Long, R.** 2001. Smear-negative pulmonary tuberculosis in industrialized countries. Chest **120:**330–334.

32. **Lyashchenko, K., et al.** 1998. Heterogeneous antibody responses in tuberculosis. Infect. Immun. **66:**3936–3940.

33. **Ma, Y., Y. M. Wang, and T. M. Daniel.** 1986. Enzyme-linked immunosorbent assay using Mycobacterium tuberculosis antigen 5 for the diagnosis of pulmonary tuberculosis in China. Am. Rev. Respir. Dis. **134:**1273–1275.

34. **Moran, G. J., F. McCabe, M. T. Morgan, and D. A. Talan.** 1995. Delayed recognition and infection control for tuberculosis patients in the emergency department. Ann. Emerg. Med. **26:**290–295.

35. **Ormerod, L. P.** 1994. Respiratory tuberculosis, p. 73–91. *In* P. D. O. Davies (ed.), Clinical tuberculosis. Chapman and Hall Medical, London, United Kingdom.

36. **Pottumarthy, S., V. C. Wells, and A. J. Morris.** 2000. A comparison of seven tests for serological diagnosis of tuberculosis. J. Clin. Microbiol. **38:**2227–2231.

37. **Rao, V. K., E. P. Iademarco, V. J. Fraser, and M. H. Kollef.** 1999. Delays in the suspicion and treatment of tuberculosis among hospitalized patients. Ann. Intern. Med. **130:**404–411.

38. **Silva, V. M. C., G. Kanujia, M. L. Gennaro, and D. Menzies.** 2003. Factors associated with humoral response to ESAT-6, 38 kDa and 14 kDa in patients with a spectrum of tuberculosis. Int. J. Tuberc. Lung Dis. **7:**478–484.

39. **Singh, M., et al.** 1992. The Mycobacterium tuberculosis 38-kDa antigen: overproduction in *Escherichia coli*, purification and characterization. Gene **117:**53–60.

40. **Sorensen, A. L., S. Nagai, G. Houen, P. Andersen, and A. B. Andersen.** 1995. Purification and characterization of a low-molecular-mass T-cell antigen secreted by *Mycobacterium tuberculosis*. Infect. Immun. **63:**1710–1717.

41. **Vera-Cabrera, L., A. Rendon, M. Diaz-Rodriguez, V. Handzel, and A. Laszlo.** 1999. Dot blot assay for detection of antidiacyltrehalose antibodies in tuberculous patients. Clin. Diagn. Lab Immunol. **6:**686–689.

42. **Verbon, A., et al.** 1992. The 14,000-molecular-weight antigen of *Mycobacterium tuberculosis* is related to the alpha-crystalline family of low molecular weight heat shock proteins. J. Bacteriol. **174:**1352–1359.

43. **Wilkinson, R. J., et al.** 1997. Evaluation of the recombinant 38-kilodalton antigen of Mycobacterium tuberculosis as a potential immunodiagnostic reagent. J. Clin. Microbiol. **35:**553–557.

44. **World Health Organization.** 1997. WHO tuberculosis diagnostics workshop: product development guidelines, workshop report, p. 1–27. World Health Organization, Geneva, Switzerland.

45. **World Health Organization.** 2010. Global tuberculosis control. World Health Organization, Geneva, Switzerland.

46. **World Health Organization.** 2010. http://www.emro.who.int/stb/pdf/CountryProfile-tun-08.pdf. Accessed 20 April 2011.

47. **Zeiss, C. R., et al.** 1984. IgG antibody to purified protein derivative by enzyme-linked immunosorbent assay in the diagnosis of pulmonary tuberculosis. Am. Rev. Respir. Dis. **130:**845–848.