Evaluation of an In-House-Developed Radioassay Kit for Antibody Detection in Cases of Pulmonary Tuberculosis and Tuberculous Meningitis

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A radioassay for the detection of antitubercular antibody has been developed. The technique involves the addition of 125I-labeled Mycobacterium tuberculosis antigen as a tracer, diluted clinical sample (serum or cerebrospinal fluid [CSF]), and heat-inactivated Staphylococcus aureus to capture the antibody, incubation for 4 h, and quantitation of the amount of antibody present in the sample. A total of 330 serum samples from patients with pulmonary tuberculosis and 138 control serum samples from individuals who were vaccinated with M. bovis BCG and from patients with pulmonary disorders of nontubercular origin were analyzed. Also, 26 CSF samples from patients with tuberculous meningitis and 24 CSF samples as controls from patients with central nervous system disorders of nontubercular origin were analyzed. Sensitivities of 80 and 73% were observed for patients with pulmonary tuberculosis and tuberculous meningitis, respectively, and specificities of 90 and 88% were seen for the two groups of patients, respectively. The sensitivity was lower, however, for human immunodeficiency virus-infected patients coinfected with M. tuberculosis. The control population could be differentiated from the patient population. This assay is rapid and user friendly and, with its good sensitivity and specificity, should benefit the population by providing diagnoses early in the course of disease and, hence, permit the early administration of appropriate chemotherapy.

Tuberculosis, caused by Mycobacterium tuberculosis, represents a major health problem worldwide. It is estimated that almost one-third of the entire world’s population is exposed to this disease (26). In 1993, the World Health Organization declared tuberculosis a global emergency and estimated that by the start of the new millennium there would be more than 1 billion newly infected individuals and more than 17 million deaths due to this disease if control programs were not implemented properly. These control programs are dependent on good tests for early diagnosis, constant monitoring of disease status, and response to and completion of treatment.

The diagnosis of tuberculosis has never been simple, especially in children and in patients with extrapulmonary tuberculosis, from whom clinical specimens for microscopy and culture are not easily obtained. Even in patients with pulmonary tuberculosis, diagnosis by microscopy is rather insensitive and culture is time-consuming.

Since the mid-1970s radioimmunoassay and enzyme-linked immunosorbent assay (ELISA) techniques have been developed for the detection of M. tuberculosis antigen and anti-M. tuberculosis antibody from clinical specimens, and these assays have various degrees of sensitivity and specificity (1, 2, 4, 7, 8, 13, 21–25, 28, 36–39, 41, 43). Almost all the assays developed at the Laboratory Nuclear Medicine Section of the Bhabha Atomic Research Centre require the detection of both antigen and antibody from clinical samples (1, 2, 21–23, 36–38). With the added risk of coinfection with human immunodeficiency virus (HIV), the need at this time is for a test with good sensitivity and specificity for rapid and early diagnosis.

With this as the aim, we have modified the antibody test and describe here the evaluation of a single test kit for the diagnosis of tuberculosis.

**MATERIALS AND METHODS**

**Antigens.** M. tuberculosis H37Rv was grown in Youman’s synthetic medium (44) for 4 to 6 weeks. Cultures were heat inactivated by autoclaving at 120°C for 20 min, followed by sonication for extraction of antigen from the cells. The suspension was ultracentrifuged at 105,000 × g for 90 min, and the supernatant was used as the source of antigen. This sonicate antigen was iodinated with 125I by the iodogen method (16). The iodinated antigen was purified from free iodide by chromatography on a Sephadrose 6B column. The immunoreactive peak was used as the radioactive antigen or tracer.

**Antibody.** Rabbit anti-M. bovis BCG antibody (M/S Dakopatts, Copenhagen, Denmark) was commercially obtained and was used for the preparation of quality control (QC) samples.

**Solid phase.** Staphylococcus aureus (strain Cowan I) was used as the solid phase. S. aureus has protein A on its surface and can bind to immunoglobulins (immunoglobulin G [IgG]) efficiently through the Fc region. The S. aureus cultures were grown in nutrient medium for 3 to 4 days. After the cells were harvested, they were heat inactivated at 80°C for 40 min. A 10% suspension was made and was used as the solid phase.

**Assay procedure.** The antibody assay developed is a single-step assay. A total of 0.1 ml of clinical sample (a serum sample diluted 1:25 or a neat cerebrospinal fluid [CSF] sample) whose antibody levels are to be determined was incubated with 0.1 ml of an S. aureus suspension diluted 1:3 and 0.3 ml of 125I-labeled M. tuberculosis antigen. Assay control samples and QC samples were also treated in the same way as the patient samples. The reaction mixture was incubated for 4 h at room temperature with constant shaking. Subsequently, 2 ml of 0.02 M barbitone buffer (pH 7.6) was added, and the entire mixture was centrifuged at 2,000 × g for 40 min. The supernatant was aspirated, the pellet was counted in...
the standard, with concentrations ranging from 5 to 200 μg/dL.

(ii) BCG vaccination. Of the basis of the following criteria, determined by taking a detailed history: (i) patients had pulmonary tuberculosis at various stages of the disease and were at various times. Of these, 26 were diagnosed as having tuberculous meningitis on the basis of clinical presentation, and response to antibacterial treatment. COPD patients were not available for 31 patients. Only 19 patients in this group had fresh cases of tuberculosis, and diagnosis was made on the basis of a combination of tests and criteria such as X ray, skin test, and presence of a contact.

Further analysis indicated that 33 of the 330 tuberculosis patients were HIV positive. Of these, 7 patients were in group I, 16 were in group II, and 10 were in group III.

The control group comprised 138 individuals between the ages of 21 and 50 years who had no prior history of tuberculosis but who had all been vaccinated with BCG. Thirty-one of these were healthy volunteers, 75 had bronchitis leading to COPD, and 28 were asthma patients. Diagnosis of COPD and asthma was done on the basis of the criteria given earlier, such as X ray, sputum cultures, clinical presentation, and response to antibacterial treatment. COPD patients were treated with aminophylline and theophylline, and asthma patients were treated with bronchodilators.

(ii) Extrapulmonary tuberculosis. Fifty CSF samples were collected from individuals with signs and symptoms of meningitis and other neurological disorders. Of these, 26 were diagnosed as having tuberculous meningitis on the basis of the results of clinical and biochemical investigations. Twenty-four of the patients had central nervous system (CNS) disorders of nontuberculous origin.

RESULTS

Calculation. The amount of antibody in the clinical samples was determined by using the two assay controls (C1 and C2) included in the kit. Control C1 represented nonspecific binding with no antibody, and control C2 represented an anti-BCG IgG concentration of 25 μg/dL. The ratio X (as a percentage) was calculated by using the counts in C1, C2, and the unknown, as follows: ([counts in unknown − counts in control C1]/(counts in control C2 − counts in control C1)) × 100. Samples that gave values of more than 100 were considered positive.

Stability of the kit. In order to ascertain the stability of the kit, the reproducibilities of values for the QC samples in the kit were determined. The expected value of the ratio for QC Q1 was 200, and the mean observed values over a period of 7 weeks were 198 and 205 at RT and 4°C, respectively. The SD and percent coefficient of variation (CV) were 22.8 and 11.5%, respectively, at RT and 18.7 and 9.1%, respectively, at 4°C. The expected value of the ratio for the second QC, Q2, was 400, with mean values of 344 and 355 at RT and 4°C, respectively. The SD and percent coefficient of variation (CV) were 22.8 and 11.5%, respectively, at RT and 18.7 and 9.1%, respectively, at 4°C. The CVs were within the statistically acceptable range for interassay variability. These results are represented graphically in Fig. 2.

Pulmonary tuberculosis. Table 1 presents the antibody responses in patients with pulmonary tuberculosis with respect to the period of treatment given, and Table 2 presents the anti-

a gamma counter, and the antibody concentration in the samples was determined by calculating the ratio as described in the Results.

Determination of assay cutoff and assay control samples. In order to determine a cutoff limit for positivity above which the clinical samples could be classified as positive, a standard curve was constructed by using anti-BCG IgG as the standard, with concentrations ranging from 5 to 200 μg/dL, as shown in Fig. 1a. The assay was performed as described above with 77 control serum samples. These samples were taken from healthy individuals and patients with pulmonary infections of nontuberculous nature. Antibody levels were quantitated by referring to the standard curve and are expressed as micrograms per deciliter. The values obtained were calculated for the upper limit of negativity as the mean + 3 standard deviations (SDs), which was found to be 25 μg of IgG per dl, as shown in Fig. 1b. With this as the basis, two assay control samples were included in the kit and for calculation purposes. Control C1, which represented nonspecific binding, was the assay buffer with no antibody, and control C2 represented a concentration of 25 μg of anti-BCG IgG per dl in the same assay buffer.

Stability of the kit. The stability of the kit was determined for a period of 7 weeks both at room temperature (RT) and at 4°C. In this case, the kits were kept at RT and at 4°C and were evaluated at 0, 1, 2, 3, 4, and 7 weeks. QC samples Q1 and Q2 were also included to determine the reproducibility of the assay.

Sample selection. (i) Pulmonary tuberculosis. Three hundred and thirty blood samples were collected from patients attending a tuberculosis clinic. The patients had pulmonary tuberculosis at various stages of the disease and were at various stages of treatment. For all patients, the diagnosis of tuberculosis was made on the basis of the following criteria, determined by taking a detailed history: (i) presenting clinical signs and symptoms such as cough and fever; (ii) BCG vaccination status, past history of tuberculosis, or presence of a close contact with tuberculosis; (iii) routine laboratory tests which included assay of sputum for acid-fast bacilli (AFB), culture of sputum, skin test, and chest X ray; and (iv) response to antituberculosis treatment, as against specific treatment for chronic obstructive pulmonary disease (COPD) and asthma.

On the basis of the criteria described above, the patients were diagnosed as having tuberculosis and classified into the groups described below.

Group I comprised 86 patients who were positive by both smear for AFB and culture. Of these, 20 patients had fresh cases of tuberculosis, 14 had been treated for tuberculosis with antituberculosis drugs for less than 6 months, 11 had been treated for 6 months to 1 year, and 33 had been treated for more than 1 year; data on the treatment given were not available for 8 patients.

Group II comprised 126 patients who were negative by smear for AFB but positive by culture. In this group, 23 patients had fresh cases of tuberculosis, 15 had been treated for less than 6 months, 16 had been treated for 6 months to 1 year, and 57 had been treated for more than a year; data on the type of treatment given were not available for 15 patients.

Group III comprised 118 patients who were negative by both smear for AFB and culture. However, 99 of these patients had previously received a diagnosis of tuberculosis on the basis of a positive smear for AFB and culture and were receiving antituberculosis treatment. Nine patients had been treated with antituberculosis drugs for less than 6 months, 14 had been treated for 6 months to 1 year, and 45 had been treated for more than 1 year; data on the type of treatment given were not available for 31 patients. Only 19 patients in this group had fresh cases of tuberculosis, and diagnosis was made on the basis of a combination of tests and criteria such as X ray, skin test, and presence of a contact.
body responses in patients with HIV and *M. tuberculosis* coinfection. It was observed that 14 of the 138 control samples had antibody levels above the ratio cutoff value of 100, contributing to a rate of nonspecific detection of 10% and a specificity of 90%.

Group I comprised 86 patients who were positive by both smear and culture and had 69 patients who had high antibody ratios, giving the test a sensitivity of 80.2% and a positive predictive value of 83.1%. Of the 7 HIV-positive patients in this group, 4 patients had high titers of antitubercular antibody. Group II had 126 patients who were negative by smear but positive by culture. Of these, 92 were positive for antibody, giving the test a sensitivity of 73% and a positive predictive value of 86.8%. Six of the 16 patients in this group were positive for antibody, giving the test a sensitivity of 60.2% and a positive predictive value of 83.5%. Three of the 10 HIV-positive patients in this group also had high antibody levels.

**Extrapulmonary tuberculosis.** Table 4 represents the percent positivity of antibody detection in patients with tuberculosis meningitis. Of the 26 patients with clinically proven tuberculous meningitis, 19 had high antibody levels by this test, giving a sensitivity of 73%. A false-positivity rate of 12% was collection and those who had not received any treatment at the time of sample collection (fresh cases). The test showed a sensitivity of 62.9% and a positive predictive value of 73.6% for the patients with fresh cases. For the treated patients, the sensitivity was 72% and the positive predictive value was 93.2%, as depicted in Table 3.

**TABLE 1. Antibody response in pulmonary tuberculosis**

| Patient group and treatment period | No. positive | % Positive | Positive predictive value (%) |
|-----------------------------------|--------------|------------|-----------------------------|
| Group I (*n* = 86)                | 69           | 80.2       | 83.1                        |
| Fresh (*n* = 20)                  | 15           | 75.0       |                             |
| Rx* for 1–6 mo (*n* = 14)         | 14           | 100        |                             |
| Rx for 6 mo–1 yr (*n* = 11)       | 10           | 91.0       |                             |
| Rx for >1 yr (*n* = 33)           | 26           | 78.8       |                             |
| Rx data not available (*n* = 8)   | 4            | 50.0       |                             |
| Group II (*n* = 126)              | 92           | 73         | 86.8                        |
| Fresh (*n* = 23)                  | 13           | 56.5       |                             |
| Rx for 1–6 mo (*n* = 15)          | 10           | 66.6       |                             |
| Rx for 6 mo–1 yr (*n* = 16)       | 14           | 87.5       |                             |
| Rx for >1 yr (*n* = 57)           | 44           | 77.2       |                             |
| Rx data not available (*n* = 15)  | 11           | 73.3       |                             |
| Group III (*n* = 118)             | 71           | 60.2       | 83.5                        |
| Fresh (*n* = 19)                  | 11           | 58.0       |                             |
| Rx for 1–6 mo (*n* = 9)           | 7            | 77.7       |                             |
| Rx for 6 mo–1 yr (*n* = 14)       | 10           | 71.4       |                             |
| Rx for >1 yr (*n* = 45)           | 27           | 60.0       |                             |
| Rx data not available (*n* = 31)  | 16           | 51.6       |                             |
| Control (*n* = 138)               | 14           | 10.0       |                             |

*Rx, treatment.*

**FIG. 2.** Stability of the kit at RT (a) and at 4°C (b). ●, C1; ■, C2; ▲, QC1; ◆, QC2.

**TABLE 2. Antibody responses in patients with HIV and *M. tuberculosis* coinfection**

| Patient group and treatment period | No. positive | % Positive |
|-----------------------------------|--------------|------------|
| Group I (*n* = 7)                 | 4            | 57.1       |
| Fresh (*n* = 1)                   | 0            |            |
| Rx* for 1–6 mo (*n* = 0)          | 0            |            |
| Rx for 6 mo–1 yr (*n* = 1)        | 1            |            |
| Rx for >1 yr (*n* = 3)            | 2            |            |
| Rx data not available (*n* = 2)   | 1            |            |
| Group II (*n* = 16)               | 6            | 37.5       |
| Fresh (*n* = 4)                   | 1            |            |
| Rx for 1–6 mo (*n* = 4)           | 1            |            |
| Rx for 6 mo–1 yr (*n* = 2)        | 2            |            |
| Rx for >1 yr (*n* = 5)            | 2            |            |
| Rx data not available (*n* = 1)   | 0            |            |
| Group III (*n* = 10)              | 3            | 30.0       |
| Fresh (*n* = 3)                   | 1            |            |
| Rx for 1–6 mo (*n* = 1)           | 0            |            |
| Rx for 6 mo–1 yr (*n* = 2)        | 1            |            |
| Rx for >1 yr (*n* = 3)            | 1            |            |
| Rx data not available (*n* = 1)   | 0            |            |

*Rx, treatment.*

Group III comprised 118 patients who were negative by smear and culture and had 69 patients who had high antibody levels, giving the test a sensitivity of 60.2% and a positive predictive value of 83.5%. Three of the 10 HIV-positive patients in this group also had high antibody levels. The detailed analysis of each subgroup is presented in Tables 1 and 2.

Figure 3 provides a graphical representation of the response ratios for the different groups of patients with pulmonary tuberculosis. Compared to the antitubercular antibody levels in the control group, the patient groups had high levels of antitubercular antibody.

The patient population was further classified as those who had received antituberculous treatment at the time of sample collection and those who had not received any treatment at the time of sample collection (fresh cases). The test showed a sensitivity of 62.9% and a positive predictive value of 73.6% for the patients with fresh cases. For the treated patients, the sensitivity was 72% and the positive predictive value was 93.2%, as depicted in Table 3.

**Extrapulmonary tuberculosis.** Table 4 represents the percent positivity of antibody detection in patients with tuberculosis meningitis. Of the 26 patients with clinically proven tuberculous meningitis, 19 had high antibody levels by this test, giving a sensitivity of 73%. A false-positivity rate of 12% was collection and those who had not received any treatment at the time of sample collection (fresh cases). The test showed a sensitivity of 62.9% and a positive predictive value of 73.6% for the patients with fresh cases. For the treated patients, the sensitivity was 72% and the positive predictive value was 93.2%, as depicted in Table 3.

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observed for the control group, giving a specificity of 88% and a positive predictive value of 91%.

Figure 4 is a graphical representation of the antibody response ratios for patients with CNS disorders of tuberculous and nontuberculous origin.

**DISCUSSION**

The need for a serological test for the diagnosis of tuberculosis is emphasized on the grounds of potential benefits for rapid and differential diagnosis, particularly in culture- and/or smear-negative patients. During bacterial diseases like tuberculosis, the immune response leads to a rise in the titers of antibodies against different antigenic determinants of the organisms, and hence, the specific antibodies present may range from those that are species specific to those that share certain common antigenic determinants with the causative organisms. The antibody response in tuberculosis has been studied for a long time (1, 2, 4, 7, 13, 17, 23–25, 28, 36–38, 41, 43). Although one of the major drawbacks, especially in serological tests, is the persistence of antibodies even after effective treatment, measurement of the antibody response to 38-kDa antigen has been found to be useful in assessing the effectiveness of treatment in patients with tuberculous meningitis (24). A radioimmunoassay for the detection of *M. tuberculosis* antigen and anti-*M. tuberculosis* antibodies developed at the Laboratory Nuclear Medicine Section of the Bhabha Atomic Research Centre has been tested with samples from patients with various clinical manifestations of tuberculosis (1, 2, 22, 24, 36–38). It was demonstrated that detection of antigen and antibody gave a good sensitivity and specificity for diagnosis. It was also shown that antibody detection alone could be useful for the diagnosis of tuberculous meningitis. Antigen and antibody detection in patients with pulmonary tuberculosis was done from immune complexes, not serum. In this particular study, antibody levels in serum samples from patients with pulmonary tuberculosis were measured.

The aim of the present work was to make the test user friendly and evaluate detection of antibody from serum rather than immune complexes in the case of pulmonary tuberculosis. The modified antibody assay described here is simple and rapid and involves the addition and incubation of all reagents together for 4 h at room temperature with a final centrifugation step. This not only reduces the pipetting errors and the number of centrifugation steps required but also reduces the incubation time, facilitating early diagnosis and hence the early institution of treatment.

The most significant aspect of this test was that it was useful for the diagnosis of tuberculosis in those patients for whom the rapid test for AFB was negative, which included patients from group II and group III. Although a false-positivity rate of 10% was observed for the control population, this was acceptable, as India is an area with a high prevalence of tuberculosis and the entire population is vaccinated with BCG.

Early diagnosis and early treatment of tuberculosis in patients coinfected with HIV are mandatory, as delayed therapy

| Patient | No. positive | % Positive | Positive predictive value (%) |
|---------|--------------|------------|-------------------------------|
| Fresh (n = 62) | 39 | 62.9 | 73.6 |
| Treated (n = 268) | 193 | 72.0 | 93.2 |

| Patient | No. positive | % Positive | Positive predictive value (%) |
|---------|--------------|------------|-------------------------------|
| Tuberculous meningitis (n = 26) | 19 | 73 | 86.4 |
| Controls (n = 24) | 3 | 12 |
of HIV-infected patients coinfected with tuberculosis in any of the serum samples reported that no antibody is detected by an immunochromato
test (ICT) for tuberculosis in any of the serum samples. It has been dropped from 62% for non-HIV-infected tuberculous patients to those presented in other reports, in which the sensitivity is lower than that for the entire tuberculous patient population (Table 1 and Table 2). These findings were similar to those presented in other reports, in which the sensitivity dropped from 62% for non-HIV-infected tuberculous patients to 28% for HIV-infected tuberculous patients (14). It has been reported that no antibody is detected by an immunochromatographic test (ICT) for tuberculosis in any of the serum samples of HIV-infected patients coinfected with M. tuberculosis or mycobacteria other than M. tuberculosis (30). Evaluation of the Mycodot immunodiagnostic test for the diagnosis of tuberculosis, which detects antibodies to lipoarabinomannan antigen, showed that it has very good specificity. The sensitivity of detection for HIV-negative tuberculous patients was 56% and much lower, as low as 25%, for HIV-positive patients (27). In general, serological assays have high negative values for HIV-positive individuals, low sensitivities, and low negative predictive values, which markedly decreases their utility in populations in which HIV infection is prevalent (6, 14, 27, 30).

Diagnosis of tuberculous meningitis has always been a challenge, especially in children. Data from a previous study in which the 38-kDa antigen in the ELISA system was used for the detection of antibodies in CSF samples showed sensitivities of 60% for patients proven to have tuberculosis postmortem, 80% for patients with culture-proven tuberculous meningitis, and 62.5% for patients with clinically proven tuberculous meningitis (24). The sensitivity in that study was superior to those reported elsewhere (1, 8). Use of the 19-kDa antigen for the detection of antibodies in CSF of patients with tuberculous meningitis could clearly differentiate the patient group from the control group in studies with a European population, but no such differentiation was observed in the study with an Indian population (5). The modified antibody assay mentioned here has a fairly good sensitivity of 73% for patients with tuberculous meningitis and a specificity of 88%, and above all, it could differentiate between the patient group and control group in the Indian population.

In Western countries, the prevalence of tuberculosis is low and a clear distinction between patient and control populations is possible. An ELISA with the 38-kDa antigen specific for the M. tuberculosis complex showed a sensitivity of 83% and a specificity of 99% (19). In a study with a Chinese population, however, the sensitivity of detection of IgG antibodies to the 38-kDa antigen was 64% and the specificity was 81% (12). Similarly, an ELISA performed with the Ag5 antigen to study the antibody response in patients with pulmonary tuberculosis showed a sensitivity of 84% and a specificity of 96% for populations in low-prevalence areas, while the sensitivity was 94% and the specificity was 80% for populations in high-prevalence areas (4). The same ELISA for the detection of antibodies used elsewhere showed sensitivities that varied from 49 to 89% and specificities that ranged from 88 to 98% (13, 28). The A60 antigen, which is a thermostable component of the purified protein derivative, has been used in the serodiagnosis of tuberculosis. In patients with smear-negative but culture-positive pulmonary tuberculosis, measurement of both IgM and IgG by ELISA revealed a sensitivity of 68% and a specificity of 100% (11). In the pediatric group, at a chosen specificity of 98%, anti-A60 IgG antibodies were observed in 68% of children with clinically active tuberculosis (15). In the Indian population, an ELISA with the A60 antigen for the detection of IgM antibodies in patients with pulmonary tuberculosis had a sensitivity of 77.5% and a specificity of 99% (18). In a study with a Chinese population, an ELISA showed a sensitivity of 84% and a specificity of 96% for populations in low-prevalence areas, while the sensitivity was 94% and the specificity was 80% for populations in high-prevalence areas (4). 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Previous reports have indicated that ELISA kits for the detection of antibody for the diagnosis of tuberculosis have
poor sensitivities and specificities (9, 31, 40). Clinical evaluation of the Andelisa IgG kit, which uses the A60 antigen derived from M. bovis BCG, gave a sensitivity of 48.3% and a specificity of 92%, while the Kreatech IgA kit, which uses the KP90 antigen derived from M. tuberculosis, showed a sensitivity of 49.7% and a specificity of 84% (9). Simultaneous evaluation of seven serological tests, i.e., two ICTs and five ELISAs, with the same clinical samples also showed poor to modest sensitivities that ranged from 16 to 57%. The specificities of these tests ranged from 80 to 97% with sera from the Mantoux test controls and 62 to 100% with sera from the anonymous controls (31). These results show lower sensitivities and specificities, indicating considerable overlap in antibody levels in the healthy control group and the patient group and indicating that none of the tests has utility for the diagnosis of tuberculosis. Evaluation of the validity of the ICT kit in a study with a Korean population showed a sensitivity of 73% for patients with fresh cases of tuberculosis and 87% for patients with reactivated tuberculosis, while the specificity was 88% for the healthy group and 94% for the nontuberculous patients and hospital workers (10). A similar evaluation of the ICT kit in Madagascar showed sensitivities of 68.2% for patients with pulmonary tuberculosis and 65.2% for patients with extrapulmonary tuberculosis and a specificity of 83.3% (33). However, evaluation of the ICT kit with the Indian population showed an overall sensitivity of 20% for patients in the first month of the disease (fresh cases) and a very low sensitivity of 4%. The test was also positive for 30% of cases of disease caused by mycobacteria other than M. tuberculosis, indicating cross-reactivity. The overall specificity of the test was 89% (30). The ELISA kit for the detection of IgG antibodies against tuberculous glycolipid antigen containing cord factor (TBGL Ag) had a sensitivity of 87% and a specificity of 100%. The other findings included in the report were an increase in the antibody titers after the start of antituberculosis chemotherapy and the persistence of antibodies in the circulation even after the completion of treatment (42). The ICT kit developed for detection of antibodies against the 38-kDa antigen of M. tuberculosis had an overall sensitivity of 70 to 92% and an overall specificity of 92 to 93% for patients with pulmonary tuberculosis. For patients with extrapulmonary tuberculosis, the overall sensitivity was 76% and the overall specificity was 92% (45). Results from a study conducted in the Europe demonstrated a low sensitivity of 50% but a high specificity of 100% (18). This low sensitivity will result in many false-negative results, whereby the negative predictive value will be unacceptably low even in countries with a high prevalence of tuberculosis (32). A specificity of 100% is not possible in developing countries, particularly in tropical areas where people are permanently in contact with various pathogens and develop cross-reacting antibodies, resulting in poor test specificities. Hence, every new serodiagnostic test should be validated with controls from countries where the test would be applied (32).

In this context a cutoff of 100 was determined on the basis of the values obtained for the control population, which included contacts and patients with nontuberculous disease. It was observed, however, that if the cutoff were raised to 125, the specificity would go up to 97% and the sensitivities would be 69.7, 66.6, and 51.6%, for groups I to III, respectively, as shown in Table 1, with a positive predictive value of 94%. Thus, it is possible to alter the cutoff and obtain appropriate sensitivity, specificity, and predictive values according to the prevalence of the disease in different areas.

The antibody test kit described here involves very few steps in terms of the addition of reagents and a shorter incubation time, which make it user friendly. Above all it has a good sensitivity and a good specificity. This kit should benefit the population by providing diagnoses early in the course of disease and, hence, permitting the early administration of appropriate chemotheraphy.

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