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# A Comparison of Seven Tests for Serological Diagnosis of Tuberculosis

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**Seven serological tests, two immunochromatographic tests, ICT Tuberculosis and RAPID TEST TB, and five enzyme-linked immunosorbent assays, TUBERCULOSIS IgA EIA, PATHOZYME-TB complex, PATHOZYME-MYCO IgG, PATHOZYME-MYCO IgA, and PATHOZYME-MYCO IgM, were evaluated simultaneously with 298 serum samples from three groups of individuals: 44 patients with active tuberculosis, 204 controls who had undergone the Mantoux test (89 Mantoux test-positive and 115 Mantoux test-negative controls), and 50 anonymous controls. The sensitivities of the tests with sera from patients with active tuberculosis were poor to modest, ranging from 16 to 57%. All the tests performed equally with sera from subgroups of those with active tuberculosis, those with pulmonary (33 patients) versus extrapulmonary (11 patients) disease, and those who were smear positive (24 patients) versus smear negative (12 patients) ($P > 0.05$). The specificities of the tests ranged from 80 to 97% with sera from the Mantoux test controls and 62 to 100% with sera from the anonymous controls. The TUBERCULOSIS IgA EIA had the highest sensitivity (57%) with sera from patients with active tuberculosis, with a high specificity of 93% with sera from the Mantoux test controls, but a very poor specificity of 62% with sera from the anonymous controls. Overall, ICT Tuberculosis followed by PATHOZYME-MYCO IgG had the best performance characteristics, with sensitivities of 41 and 55%, respectively, with sera from patients with active tuberculosis and specificities of 96 and 89%, respectively, with sera from the Mantoux test controls and 88 and 90%, respectively, with sera from the anonymous controls. By combining all the test results, a maximum sensitivity of 84% was obtained, with reciprocal drops in specificities to 55 and 42% for the Mantoux test controls and anonymous controls, respectively. The best combination was that of ICT Tuberculosis and PATHOZYME-MYCO IgG, with a sensitivity of 66% and a specificity of 86% for the Mantoux test controls and a sensitivity and specificity of 78% for the anonymous controls. While a negative result by any one of these tests would be useful in helping to exclude disease in a population with a low prevalence of tuberculosis, a positive result may aid in clinical decision making when applied to symptomatic patients being evaluated for active tuberculosis.**

Tuberculosis has been declared a global emergency. The mainstay for its control is the rapid and accurate identification of infected individuals. The simplest rapid method is the detection of acid-fast bacilli by microscopy. However, 40 to 60% of patients with pulmonary disease and ~75% of patients with extrapulmonary disease are smear negative, and in this situation even contemporary culture methods take several weeks to become positive (7, 11, 12). Therefore, a number of alternative diagnostic tests that use molecular, chromatographic and immunological methods have been developed. While molecular methods overcome the insensitivity of the smear method and the time required for culture, they depend upon retrieval of a specimen from the site of infection. This is often difficult in cases of tuberculosis in children and in some cases of extrapulmonary disease.

Immunological methods use the specific humoral or cellular responses of the host to infer the presence of infection or disease. They do not require a specimen from the site of infection. Numerous serological tests that use various antigens, such as secreted and heat shock proteins, lipopolysaccharides, and peptides, have been developed (2). These tests use various modifications of enzyme-linked immunosorbent assay (ELISA) or immunochromatographic methods to detect different antibody classes. Only rarely has more than one serological test been evaluated with sera from the same group of individuals (2, 4, 10). We have evaluated seven serological tests to determine their performances with sera from three groups of individuals.

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## MATERIALS AND METHODS

**Study population.** Over 16 months, May 1997 to September 1998, 298 individuals were evaluated. All were human immunodeficiency virus negative. They comprised three groups: 44 patients with active tuberculosis, 204 controls who had undergone the Mantoux test, and 50 anonymous controls. The patients had a median age of 37 years (age range, 15 to 81 years), and 22 (50%) were males. Of the 44 patients with active tuberculosis, 33 (75%) had pulmonary disease and 11 (25%) had extrapulmonary disease. Extrapulmonary diseases included lymphadenitis (four patients), disseminated disease (four patients), pleural disease (two patients), and peritoneal tuberculosis (one patient). Thirty-six (82%) patients had culture-proven disease, with 24 being smear positive and 12 being smear negative. For the eight culture-negative patients the diagnosis was made on the basis of clinical, radiographic, and histologic findings and response to antituberculous treatment. Sera were obtained from these patients within days of their admission to the tuberculosis ward and from almost all patients before initiation of antituberculosis treatment.

Mantoux test controls included 146 immigrants from countries with a high prevalence of tuberculosis (118 New Zealand quota refugees and 28 asylum seekers undergoing screening for infectious diseases), 38 health care workers undergoing employment screening at the occupational health clinics of Auckland and Green Lane Hospitals, and 20 microbiology staff from these hospitals and a community laboratory in Auckland. This group had a median age of 30 years (age range, 9 to 72 years), and 95 (47%) were males. A total of 193 of 204 (94%) of the Mantoux test controls had received the *Mycobacterium bovis* BCG vaccine. The Centers for Disease Control and Prevention interpretative criteria for positive Mantoux test results were followed (3). A total of 115 (56%) individuals were Mantoux test negative, and on the basis of clinical and radiological findings no individuals in this group had active tuberculous disease. The anonymous control group included sera from 50 individuals; these samples were selected at random from blood samples submitted for routine biochemistry testing from general adult medical and surgical wards. Sera from patients from our tuberculosis ward were not included. As the prevalence of tuberculosis in these patients

TABLE 1. Performances of seven serological tests with sera from patients with active tuberculosis

| Test | No. (%) seropositive individuals among those with active tuberculosis | | | *P* value for individuals with pulmonary versus extrapulmonary disease[a] | No. (%) of seropositive individuals among those with active tuberculosis | | *P* value for smear-positive versus smear-negative individuals[a] |
|---|---|---|---|---|---|---|---|
| | Total ($n = 44$) | Pulmonary ($n = 33$) | Extrapulmonary ($n = 11$) | | Smear positive ($n = 24$) | Smear negative ($n = 12$) | |
| ICT Tuberculosis | 18 (41) | 16 (49) | 2 (18) | 0.08 (NS) | 11 (46) | 4 (33) | 0.47 (NS) |
| RAPID TEST TB | 11 (25) | 9 (27) | 2 (18) | 0.55 (NS) | 7 (29) | 3 (25) | 0.79 (NS) |
| TUBERCULOSIS IgA EIA | 25 (57) | 19 (58) | 6 (55) | 0.86 (NS) | 13 (54) | 7 (58) | 0.81 (NS) |
| PATHOZYME-TB complex | 7 (16) | 5 (15) | 2 (18) | 0.81 (NS) | 4 (17) | 2 (17) | 1.00 (NS) |
| PATHOZYME-MYCO IgG | 24 (55) | 19 (58) | 5 (46) | 0.48 (NS) | 15 (63) | 6 (50) | 0.47 (NS) |
| PATHOZYME-MYCO IgA | 18 (41) | 15 (46) | 3 (27) | 0.29 (NS) | 11 (46) | 3 (25) | 0.23 (NS) |
| PATHOZYME-MYCO IgM | 8 (18) | 7 (21) | 1 (9) | 0.37 (NS) | 5 (21) | 2 (17) | 0.77 (NS) |

[a] Significantly different results are indicated by a *P* value of $\leq 0.05$. NS, not statistically different.

is very low, for the purpose of analysis they were regarded as not having active disease.

Sera were stored at −70°C. All the kits were tested simultaneously. Testing was performed according to each manufacturer's instructions.

**Immunochromatographic tests.** The ICT Tuberculosis diagnostic kit was provided by ICT DIAGNOSTICS (Balgowlah, New South Wales, Australia). The principle of this test has been described previously (5). In brief, five highly purified antigens (including one of 38 kDa) secreted by *Mycobacterium tuberculosis* during active infection are immobilized in four lines on the test strip. The test detects the presence of immunoglobulin G (IgG) antibodies to these antigens. A total of 30 μl of serum is added to a blue pad and then diffuses along the test strip. When the test card is closed, anti-human IgG attached to colloidal gold particles binds to any bound human IgG antibodies, producing one or more pink lines. The presence of one or more pink lines in the strip's test area is considered a positive test result.

The RAPID TEST TB, provided by QUORUM DIAGNOSTICS (Vancouver, British Columbia, Canada), is a one-step colored immunochromatographic assay. It detects antibodies to the recombinant 38-kDa antigen from *M. tuberculosis* expressed in and purified from *Escherichia coli*. A total of 100 μl of serum is added to the reaction tube, and the test strip is placed into the tube, which is then capped. After 15 min of incubation, the presence of two colored bands is considered a positive test result.

**ELISAs.** TUBERCULOSIS IgA EIA, provided by KREATECH Diagnostics (Amsterdam, The Netherlands), detects IgA antibody to a mycobacterial Kp90 immuno-cross-reactive antigenic compound (ImCRAC). A total of 100 μl of diluted (1:400) serum was distributed in microtiter wells, and the plate was incubated at 37°C in a dark humid environment for 60 min. Unbound serum components were removed by washing with a buffer solution. The wells were subsequently incubated with 100 μl of peroxidase-labeled anti-human IgA conjugate at 37°C in a dark humid environment for 60 min. After another wash cycle, 100 μl of peroxidase substrate, tetramethylbenzidine containing hydrogen peroxide, was added to the wells. The colorimetric reaction proceeded in the dark for 30 min at room temperature (20 to 25°C) until 100 μl of stop agent was added. The absorbance values at 450 nm were recorded with an automatic reader system. The cutoff optical density (OD) was derived from the mean of the three cutoff control OD readings (as recommended by the manufacturer). The ratio of the OD for the unknown serum sample to the cutoff OD was used to interpret the results.

The PATHOZYME-TB complex test kit was provided by OMEGA DIAGNOSTICS LIMITED (Alloa, Scotland). The kit detects serum IgG antibody to a recombinant 38-kDa antigen from *M. tuberculosis* expressed in and purified from *E. coli*. This kit is specific for the diagnosis of disease due to *M. tuberculosis* complex. The procedures were similar to those described for TUBERCULOSIS IgA EIA, except that the sera were diluted 1:50, the second incubation was for 30 min rather than 60 min, the first and second incubations were not in a dark humid environment, and the third incubation was in the dark at 37°C for 15 min. Three standards (with 2, 4, and 16 serounits/ml) were provided for the generation of a semilogarithmic reference curve. Because the sera were diluted 1:50, the units extrapolated from the reference curve were multiplied by 50 to obtain serounits for result interpretation.

The individual PATHOZYME-MYCO IgG, IgA, and IgM assay kits were provided by OMEGA DIAGNOSTICS LIMITED. The three assays measure the levels in serum of IgG, IgA, and IgM antibodies, respectively, to two antigens; lipoarabinomannan (LAM) and recombinant 38-kDa antigen. These kits detect infection due to *Mycobacterium* species. The procedures were identical to those described for PATHOZYME-TB complex except that the sera were diluted 1:100 rather than 1:50 and all three incubations were at room temperature. For the IgG and IgA assays three standards (with 2, 4, and 16 serounits/ml) were provided for generation of a semilogarithmic reference curve. Because the sera were diluted 1:100, the units extrapolated from the reference curve were multiplied by 100 to obtain serounits for result interpretation. For the IgM assay, low- and high-positive control sera were provided. The OD of the low-positive control was used for the interpretation of the results.

**Statistical analysis.** Sensitivities, specificities, and predictive values were calculated by standard methods. Sensitivity was defined as the ability to detect cases of active tuberculosis. Specificity was defined as the ability to be negative for the Mantoux test and anonymous controls, who, for the purposes of analysis, were considered to be free of active tuberculosis. The differences in the performances of the tests were analyzed by the $\chi^2$ test. Positive and negative predictive values were calculated for different prevalences of disease: 11 per 100,000 population (0.01%) as the overall rate of new cases of tuberculosis in New Zealand in 1995; 125 per 100,000 population (0.125%) as the highest rate of tuberculosis in those of other ethnic background, mainly Southeast Asian people with tuberculosis; and 76% as the prevalence of disease among patients being evaluated for tuberculosis in our tuberculosis ward (8, 9).

## RESULTS

**Performance of the serological tests with sera from patients with active tuberculosis.** For the 44 patients with active tuberculosis, the sensitivities of the assays ranged from 16 to 57% (Table 1). For the 33 patients with pulmonary tuberculosis, the sensitivities of the assays ranged from 15 to 58%, with the lowest being for PATHOZYME-TB complex (15%) and the highest being for TUBERCULOSIS IgA EIA and PATHOZYME-MYCO IgG (58% each). For the 11 patients with extrapulmonary disease, the sensitivity ranged from 9 to 55%, with the lowest being for PATHOZYME-MYCO IgM (9%) and the highest being for TUBERCULOSIS IgA EIA (55%). For the 24 smear-positive patients, the sensitivity ranged from 17 to 63%, with the lowest being for PATHOZYME-TB complex (17%) and the highest being for PATHOZYME-MYCO IgG (63%). For the 12 smear-negative patients, the sensitivity ranged from 17 to 58%, with the lowest being for PATHOZYME-TB complex and PATHOZYME-MYCO IgM (17% each) and the highest being for TUBERCULOSIS IgA EIA (58%). The differences between the performances of the assays were not statistically significant between the groups, analyzed as either pulmonary versus extrapulmonary cases or smear-positive versus smear-negative cases ($P > 0.05$) (Table 1).

**Performances of the seven tests with sera from the control groups.** For the 204 Mantoux test controls, the test positivity rate ranged between 3 and 20%, with the lowest being for PATHOZYME-TB complex (3%) and the highest being for PATHOZYME-MYCO IgM (20%) (Table 2). Mantoux test-positive individuals had higher positivity rates than the Mantoux test-negative individuals (except with the PATHOZYME-MYCO IgG and IgM kits), and the difference was statistically significant for RAPID TEST TB, TUBERCULOSIS IgA EIA, PATHOZYME-TB complex, and PATHOZYME-MYCO IgA ($P < 0.05$). For the 50 anonymous controls, the test positivity rate ranged between 0 and 38%, with the lowest being

TABLE 2. Performances of seven serological tests with sera from those with known Mantoux test results and anonymous controls

| Test | No. (%) of seropositive Mantoux test controls | | | *P* value for Mantoux test-positive versus -negative controls[a] | No. (%) of seropositive anonymous controls ($n$ = 50) |
|---|---|---|---|---|---|
| | Total ($n$ = 204) | Mantoux test positive ($n$ = 89) | Mantoux test negative ($n$ = 115) | | |
| ICT Tuberculosis | 9 (4) | 5 (6) | 4 (4) | 0.15 (NS) | 6 (12) |
| RAPID TEST TB | 26 (13) | 15 (17) | 11 (10) | 0.03 (S) | 7 (14) |
| TUBERCULOSIS IgA EIA | 14 (7) | 10 (11) | 4 (4) | 0.03 (S) | 19 (38) |
| PATHOZYME-TB complex | 7 (3) | 6 (7) | 1 (1) | 0.01 (S)[b] | 2 (4) |
| PATHOZYME-MYCO IgG | 22 (11) | 9 (10) | 13 (11) | 0.7 (NS) | 5 (10) |
| PATHOZYME-MYCO IgA | 30 (15) | 18 (20) | 12 (10) | 0.01 (S) | 14 (28) |
| PATHOZYME-MYCO IgM | 40 (20) | 17 (19) | 23 (20) | 0.48 (NS) | 0 (0) |

[a] Significantly different results are indicated by a *P* value of ≤0.05. S, statistically significant; NS, not statistically different.
[b] Fifty percent of the cells in the frequency table had expected counts of less than 5. This result should therefore be interpreted cautiously.

for PATHOZYME-MYCO IgM (0%) and the highest being for TUBERCULOSIS IgA EIA (38%) (Table 2).

**Sensitivities, specificities, and predictive values of the serological tests.** For the 44 patients with active tuberculosis, the sensitivities of the serological tests were poor to moderate, ranging from 16 to 57% (Table 3). The specificities of the tests for the Mantoux test controls ranged from 80 to 97% (Table 3). The specificities of the tests for the anonymous controls were poor, ranging from 62 to 100%, and for five tests it was ≤90% (Table 3). Because the Mantoux test group was the larger of the two control groups and was known definitely not to contain individuals with active tuberculosis, the predictive values were calculated by using the data for the Mantoux test control group. All seven tests had excellent negative predictive values (99.9%) at either a 0.01% prevalence of tuberculosis (the overall rate of tuberculosis in New Zealand) or a 0.1% prevalence of tuberculosis (the highest rate of tuberculosis analyzed in an ethnic group) (8), while the positive predictive value was very poor (≤1%) (Table 4). At a prevalence of 76% (prevalence of disease in patients admitted for evaluation to our tuberculosis ward) (9), all tests had good positive predictive values, which ranged from 74 to 97%, but had poor negative predictive values (≤41%) (Table 4).

**Combination of results obtained by the seven tests.** Different combinations of results obtained by the immunochromatographic tests and the ELISAs were analyzed to maximize the sensitivity (Table 5). A maximum sensitivity of 84% was obtained when the results of all seven tests were combined, but there were decreases in specificities for the Mantoux test controls and the anonymous controls to 55 and 42%, respectively (Table 5). The best combination was that of ICT Tuberculosis and PATHOZYME-MYCO IgG, with a sensitivity of 66% and with specificities of 86 and 78% for the Mantoux test and anonymous controls, respectively. Combination of results of the PATHOZYME-MYCO IgG, IgA, and IgM panel of tests also increased the sensitivity to 66%, but the specificities decreased for the Mantoux test and anonymous controls to 67 and 70%, respectively (Table 5).

TABLE 3. Sensitivities and specificities of the seven serological tests

| Test | No. positive (% sensitivity) among those with active tuberculosis ($n$ = 44) | No. negative (% specificity) | |
|---|---|---|---|
| | | Mantoux test controls ($n$ = 204) | Anonymous controls ($n$ = 50) |
| ICT Tuberculosis | 18 (41) | 195 (96) | 44 (88) |
| RAPID TEST TB | 11 (25) | 178 (87) | 43 (86) |
| TUBERCULOSIS IgA EIA | 25 (57) | 190 (93) | 31 (62) |
| PATHOZYME-TB complex | 7 (16) | 197 (97) | 48 (96) |
| PATHOZYME-MYCO IgG | 24 (55) | 182 (89) | 45 (90) |
| PATHOZYME-MYCO IgA | 18 (41) | 174 (85) | 36 (72) |
| PATHOZYME-MYCO IgM | 8 (18) | 164 (80) | 50 (100) |

## DISCUSSION

More than 100 years ago, in 1898, Arloing reported the first serodiagnostic test for tuberculosis, an agglutination test, just 16 years after Koch's identification of the tubercle bacillus. For the next eight decades numerous serological techniques were evaluated, but they gave poor results due to the cross-reactive nature of the antigens used. Since the introduction of ELISA in 1972 and the availability of monoclonal antibodies as well as purified antigens, the serological diagnosis of tuberculosis has become more promising (6, 7). The 38-kDa antigen, a phosphate-binding protein, has been identified as the immunodominant antigen in smear-positive pulmonary tuberculosis and a potential reagent for use in screening for infectious tuberculosis (2, 13). This antigen is common to six of the seven tests that we evaluated, the exception being the TUBERCULOSIS IgA EIA, which uses the Kp90 ImCRAC antigen.

The sensitivities and specificities of the seven tests varied widely, with sensitivities ranging from ~20 to 60% and specificities ranging from ~65 to 100%. The sensitivities of the tests were generally lower than those obtained by other investigators (4, 5, 14; B. Lopez, N. Masciotra, and L. Barrera, Tubercle Lung Dis. **77**[Suppl. 2]**:**119 [abstract], 1996). Evaluation of a previous version of ICT Tuberculosis (which contained only the 38-kDa antigen) with sera from 152 patients with active pulmonary tuberculosis in China by Cole et al. (5) showed a sensitivity of 89% for smear-positive patients and a sensitivity of 74% for smear-negative patients, with a specificity of 93% (5). For the TUBERCULOSIS IgA EIA, various results have been reported (1, 4). While Alifano et al. (1) reported a sensitivity of 70% for 88 patients with microbiologically confirmed pulmonary tuberculosis, with a specificity of 92%, Chiang et al. (4), using the cutoff recommended by the manufacturer, reported a sensitivity of 80% for 312 patients with active pulmonary tuberculosis but a very low specificity (42%) which was improved to 66% by adjusting the cutoff, but with a reduction in sensitivity to 63%. Lopez et al. (Tubercle Lung Dis. **77**[Suppl. 2]**:**119 [abstract], 1996) evaluated two of the PATHOZYME ELISAs, PATHOZYME-TB complex and PATHOZYME-MYCO IgG, with sera from 26 smear-negative patients and reported sensitivities of 40 and 42%, respectively, with specificities of 96%.

TABLE 4. Positive and negative predictive values for the seven serological tests at different prevalences of tuberculosis

| Test | Prevalence of 0.01% (general New Zealand population)[a] | | Prevalence of 0.1% (group with highest prevalence in New Zealand, predominantly Southeast Asian)[a] | | Prevalence of 76% (tuberculosis ward, Green Lane Hospital)[b] | |
|---|---|---|---|---|---|---|
| | PPV[c] (%) | NPV[d] (%) | PPV (%) | NPV (%) | PPV (%) | NPV (%) |
| ICT Tuberculosis | 0.1 | 99.9 | 1 | 99.9 | 97 | 34 |
| RAPID TEST TB | 0.02 | 99.9 | 0.2 | 99.9 | 86 | 27 |
| TUBERCULOSIS IgA EIA | 0.08 | 99.9 | 0.8 | 99.9 | 96 | 41 |
| PATHOZYME-TB complex | 0.05 | 99.9 | 0.5 | 99.9 | 94 | 27 |
| PATHOZYME-MYCO IgG | 0.05 | 99.9 | 0.5 | 99.9 | 94 | 38 |
| PATHOZYME-MYCO IgA | 0.03 | 99.9 | 0.3 | 99.9 | 90 | 31 |
| PATHOZYME-MYCO IgM | 0.009 | 99.9 | 0.09 | 99.9 | 74 | 24 |

[a] Data are from reference 8.
[b] Datum is from reference 9.
[c] PPV, positive predictive value.
[d] NPV, negative predictive value.

A higher rate of seropositivity for the smear-positive group compared to that for the smear-negative group has been attributed to the higher bacillary loads in smear-positive patients, resulting in a greater exposure to antigen and thus a more vigorous antibody response (1, 6). Similar to other investigators, we observed generally higher sensitivities for the smear-positive group than for the smear-negative group and for the pulmonary infection group than for the extrapulmonary infection group (6, 10, 14). These differences, however, did not reach statistical significance for either group. The assays could therefore be useful in diagnosing more difficult forms of tuberculosis, i.e., extrapulmonary and smear-negative tuberculosis. However, as the number of patients in our study was small, a larger number needs to be studied to confirm our findings.

Mantoux test-positive controls were more often antibody positive than the Mantoux test-negative controls. This was statistically significant for four of the seven tests. Most of the Mantoux test controls (~95%) had received the *M. bovis* BCG vaccine in the past. Three of the seven antigens used in the tests, the 38-kDa, Kp90 ImCRAC, and LAM antigens, are not specific for *M. tuberculosis* and are present in *M. bovis* BCG as well (1, 5, 6). While *M. bovis* BCG expresses the 38-kDa antigen, it is present at only 1/10 of the concentration at which it is found in *M. tuberculosis*; thus, the antibody response from BCG vaccination is neither expected to be very high nor persist for very long (5). Similarly, IgA antibodies to Kp90 are reported to occur only in patients with tuberculosis (1). However, Zhou et al. (14) reported a 9% seropositivity rate for healthy BCG-vaccinated children when their sera were tested by the ICT Tuberculosis test. Those investigators speculated that while it could be due to residual antibody to recent BCG vaccinations in children, it was more likely to be due to primary tuberculosis infection (14). Cole et al. (5) reported that 1 of 30 healthy BCG-vaccinated adult controls had a faint positive response by the ICT Tuberculosis test and attributed it to the possibility of a subclinical infection (5). Similarly, Alifano et al. (1) observed the production of anti-Kp90 IgA in healthy Mantoux test-positive and -negative subjects and hypothesized that it could be due to clinically silent infection with environmental mycobacteria, as Kp90 is not species specific (1).

TABLE 5. Sensitivities and specificities of combinations of serological tests

| Test[a] | % Sensitivity among those with active tuberculosis ($n$ = 44) | % Specificity | |
|---|---|---|---|
| | | Mantoux test controls ($n$ = 204) | Anonymous controls ($n$ = 50) |
| ICT and PMG | 66 | 86 | 78 |
| ICT and RTB | 48 | 83 | 76 |
| PMG, PMA, and PMM | 66 | 67 | 70 |
| ICT, RTB, and PMG | 73 | 75 | 68 |
| ICT, RTB, PTB, PMG, PMM, and PMA | 75 | 57 | 54 |
| ICT, RTB, PTB, PMG, PMM, PMA, and KIA | 84 | 55 | 42 |

[a] ICT, ICT Tuberculosis; RTB, RAPID TEST TB; KIA, TUBERCULOSIS IgA EIA; PTB, PATHOZYME-TB complex; PMG, PATHOZYME-MYCO IgG; PMA, PATHOZYME-MYCO IgA; PMM, PATHOZYME-MYCO IgM.

The seropositivity rates of the tests for the anonymous controls, i.e., general medical and surgical adult patients, were generally higher than those for the Mantoux test controls and ranged from 0 to 40%. Positivity rates of 9 and 12% for controls with nontuberculous lung diseases have been reported for ICT Tuberculosis (5, 14). This has been attributed to the fact that damaged lung tissues in patients with these conditions allow the entry of invasive organisms like tubercle bacilli into the tissues (5). Alifano et al. (1) reported a 14% false-positivity rate for TUBERCULOSIS IgA EIA for controls with nontuberculous lung diseases and attributed it to the disorders in the immune response that occur in the course of these diseases. Similarly, Chiang et al. (4) reported high positivity rates of 53 and 49% for the fibrocalcified tuberculosis group and 19 and 34% for the joint group of healthy controls and nontuberculous pulmonary diseases for the 38-kDa IgG ELISA and the TUBERCULOSIS IgA EIA, respectively.

The diagnostic value of a given test in clinical practice depends on its positive and negative predictive values (4). These values vary markedly with the prevalence of the disease in a given community (7). With the very low prevalence of tuberculosis in New Zealand, i.e., 0.01% in the general population and 0.1% in the subgroup with the highest rate, any one of the seven tests would provide a good negative predictive value for exclusion of the disease, while a positive result would be unhelpful. In contrast, if used with patients admitted to our tuberculosis ward for evaluation, among whom the prevalence of active disease in those being evaluated for active tuberculosis is 76% (9), the high positive predictive values of tests would make a positive test result useful in strengthening the clinical suspicion, but a negative result would be less useful. Thus, these tests could potentially be used for those subgroups of patients with tuberculosis from whom specimens are hard to

obtain, i.e., those with extrapulmonary and childhood tuberculosis and smear-negative patients, to aid in clinical decision making.

Investigators have recommended improving the performance values of the tests by either adjusting the cutoff values or combining the results of different tests (2, 4, 11). Because two of the tests that we evaluated were immunochromatographic assays, we tried using different combinations of the seven tests to maximize the sensitivity. Even by combining the results of all the tests, i.e., tests with seven antigens in various combinations, and detecting three different classes of antibodies, a sensitivity of 100% could not be achieved, and a maximum sensitivity of 84% with a substantial loss of specificity to ≤55% was obtained. The sensitivities of tests with antibodies to single antigens have been reported to be ~75% at best for patients with smear-positive tuberculosis (2). Even by combining serological responses to several antigens, sensitivity is rarely said to exceed 90%, due to either a lack of immune responsiveness based on the HLA phenotype, a predominant Th1 (T-helper) immune response, or the formation of immune complexes that make the antibody unavailable (2).

Evaluation of the seven tests simultaneously with sera from the same population allowed us to compare the tests directly. All the tests were user friendly, and the results were obtained within 6 h. Unlike the five ELISAs, which required the necessary equipment and some technical expertise, the two immunochromatographic tests, ICT Tuberculosis and RAPID TEST TB, required no special equipment and little or no technical skill and answers were obtained within 30 min.

**Conclusion.** We evaluated seven serological tests with sera from three groups of individuals: patients with active tuberculosis and two control groups. Even the best of the tests had a modest sensitivity of ≤60% for the detection of active tuberculosis. The tests had good specificities for the Mantoux test controls but poor specificities for the anonymous controls. The diagnostic values of these tests depend on the context of their use. While a negative result would be useful in excluding disease in a population with a low prevalence of tuberculosis, a positive result could potentially aid in clinical decision making when used with sera from a group of selected symptomatic patients when there is a moderate to high degree of clinical suspicion of tuberculosis.

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