Field Evaluation of a Rapid Immunochromatographic Test for Tuberculosis

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Rapid diagnostic tests for tuberculosis (TB) are needed to facilitate early treatment of TB and prevention of *Mycobacterium tuberculosis* transmission. The ICT Tuberculosis test is a rapid, card-based immunochromatographic test for detection of antibodies directed against *M. tuberculosis* antigens. The objective of the study was to evaluate the performance of the ICT Tuberculosis test for the diagnosis of active pulmonary TB (PTB) with whole blood, plasma, and serum from patients suspected of having PTB and from asymptomatic controls in a setting with a high prevalence of PTB. Seventy patients suspected of having PTB (and who were later confirmed to have or not to have PTB by use of *M. tuberculosis* culture as the "gold standard") and 42 controls were studied. Twenty-one controls were neither vaccinated with *Mycobacterium bovis* bacillus Calmette-Guérin (BCG) nor tuberculin skin test (TST) positive (group A controls), and 21 controls were TST positive and/or had previously been vaccinated with BCG (group B controls). Study subjects were drawn from one hospital and one primary health care unit in Rio de Janeiro City, Brazil. One version of the test (ICT-1) was evaluated by using whole blood, plasma, and serum samples. Sera obtained for this study were frozen and later tested with a manufacturer-modified version of the test (ICT-2). Among the patients suspected of having PTB, the sensitivities of the ICT-1 with whole blood, serum, and plasma were 83, 65, and 70%, respectively, and the specificities were 46, 67, and 56%, respectively. Among the group A controls, the specificities of ICT-1 with the three specimen types were 95, 100, and 95%, respectively. Among the group B controls, the specificities of ICT-1 with the three specimen types were 71, 86, and 86%, respectively. Among the patients suspected of having PTB, the sensitivity of ICT-2 was 70% and the specificity was 65%. Among the group A controls, the specificity of ICT-2 was 95%, and among the group B controls, the specificity of ICT-2 was 81%. With a 29% observed prevalence of PTB among patients suspected of having PTB, the positive predictive values of the ICT tests ranged from 39 to 50% and the negative predictive values ranged from 82 to 87%. The ICT Tuberculosis tests were not sufficiently predictive to warrant their widespread use as routine diagnostic tests for PTB in this setting. However, further evaluation of these tests in specific epidemiologic settings may be warranted.

Tuberculosis (TB) is the second leading cause of mortality from infectious diseases worldwide, with 2 million deaths due to TB each year (6). Microscopic examination of sputum is the only 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 (7, 8). The sensitivity of sputum smear microscopy has been reported to be lower among human immunodeficiency virus (HIV)-infected persons (5, 10). A delayed or missed diagnosis of TB contributes to *Mycobacterium tuberculosis* transmission and mortality due to TB (9, 13). A sensitive and specific diagnostic test for the rapid, point-of-care identification of patients with active TB would facilitate early treatment and prevention of transmission.

An antibody-based serological test for TB has long been sought. Such a test would be attractive because of its potential technical simplicity, rapidity, low cost, and lack of reliance on sputum (which sometimes can be difficult to obtain or uninformative). Unfortunately, most serological tests for TB have had low sensitivities and low specificities (2). Studies that use recombinant culture filtrate antigens indicate that antibody responses (at least to the antigens studied) in TB patients may be heterogeneous from patient to patient (12). Incorporation of multiple antigens in a diagnostic test may therefore increase the sensitivity of an antibody-based test. For the antibody-based tests evaluated to date, the presence of anti-*M. tuberculosis* antibodies in persons with latent *M. tuberculosis* infection, prior active TB disease, vaccination *with Mycobacterium bovis* bacillus Calmette-Guerin (BCG), or infection with mycobacteria other than *M. tuberculosis* may have contributed to the low specificities of the tests for the diagnosis of active TB.

The ICT Tuberculosis test (AMRAD Corporation, Melbourne, Victoria, Australia) is a rapid card-based test for the detection of immunoglobulin G (IgG) antibodies directed against five purified *M. tuberculosis* antigens immobilized in four lanes on a test strip and uses an anti-human IgG labeled with colloidal gold. Since the target proteins are secreted by actively growing organisms, the test has the potential to have a high degree of specificity for active TB disease. Incorporation of multiple antigens may result in a high degree of sensitivity.

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After the introduction of the first ICT Tuberculosis test (ICT-1), a manufacturer-modified version (ICT-2) was introduced. Modifications included reductions in the concentrations of proteins in bands 1, 2, and 4; a reduction in the width of the nitrocellulose strip; and reductions in the specimen pad size, conjugate pad size, and conjugate volume. ICT-1 was labeled by the manufacturer for use with serum specimens, and ICT-2 was developed to potentially include whole-blood and plasma specimens.

The purpose of the study described here was to evaluate the performance of the ICT Tuberculosis tests for the diagnosis of PTB in a setting with a high prevalence of TB.

MATERIALS AND METHODS

Study participants were drawn from the Ambulatory Unit of the Hospital Universitário Clementino Fraga Filho (HUCFF), Ilha do Fundão, and the Centro Municipal de Saúde Belizario Penna Da XVIII RA in Campo Grande, Rio de Janeiro City, Brazil, between 26 October 1999 and 30 November 1999. Approval for this study was granted by the institutional review boards of HUCFF, the Brazilian Ministry of Health, and Johns Hopkins University. Written informed consent was obtained from all study subjects. The study included 70 consecutive adult patients with symptoms or signs suggestive of active PTB (patients suspected of having PTB). Two control groups were selected from among healthy HUCFF students and staff who were enrolled in an annual TB surveillance program. Those who participate in this program receive a chest X ray and tuberculin skin test (TST) at entry and an annual evaluation that includes a symptom review for all participants and a TST for participants with a negative prior TST. Group A controls included 21 asymptomatic subjects with no history of TB or vaccination with BCG, no scar from vaccination with BCG, and a negative TST result. Group B controls included 21 asymptomatic subjects with no history of TB but with a scar from vaccination with BCG, a history of vaccination with BCG, or a positive TST result (induration, ≥10 mm). A chest X ray and a sputum specimen were not obtained solely for study purposes for the controls since all controls had a prior chest radiograph not compatible with active TB and no symptoms at the most recent annual evaluation or at study entry. Persons less than 16 years of age were excluded from the study.

Demographic and clinical data were collected for all study participants through a standardized questionnaire, interview, and physical examination. Venous blood was collected from all participants. Study participants underwent a two-step TST. An induration of ≥10 mm in the first or second step was considered a positive TST result. Patients suspected of having PTB were also given a postanterior and lateral chest X ray and were tested for HIV (by enzyme-linked immunosorbent assay with confirmation by Western blotting).

The sputa of all patients suspected of having PTB were tested for acid-fast bacilli (AFB) by the Ziehl-Neelsen technique and were cultured in Löwenstein-Jensen medium. Patients unable to expectorate sputum or for whom two consecutive specimens of expectorated sputum were smear negative for AFB underwent sputum induction with a 3% hypertonic saline solution generated with a DeVilbiss ultrasonic nebulizer (Ultra Neb 99; Home and Aid Healthcare Inc.). All specimens that were culture positive for mycobacteria were tested by standard biochemical methods to distinguish *M. tuberculosis* from other nontuberculous mycobacteria. The reference standard for the diagnosis of PTB was growth of *M. tuberculosis* in a culture of sputum. The Caracas clinical case definition (18) was used to establish a diagnosis of AIDS.

Venous blood was drawn from the study participants and placed into glass tubes without preservative or anticoagulant for separation of serum and into EDTA- or heparin-coated tubes for whole blood and separation of plasma. The serum remaining after testing by ICT-1 was frozen at −20°C for retesting at a later date by ICT-1 and ICT-2. Whole blood from seven patients suspected of having PTB was not retained for testing; plasma but not serum was obtained for seven patients suspected of having PTB, and serum but not plasma was obtained for seven other patients suspected of having TB. These participants were maintained in the study since the performance of each test with each type of specimen was evaluated separately.

ICT-1 and ICT-2 were performed and their results were interpreted according to the instructions of the manufacturer by an investigator masked to the clinical and microbiological status of the study subjects. For ICT-1, 50 μl of serum, whole blood, or plasma was tested as soon as possible on the day of collection. Each positive band was scored for location (bands 1 to 4, with band 1 at the bottom of

the card) and for intensity (0, no signal; 1, weak signal; 2, strong signal). A positive signal for any antigen was considered an overall positive ICT-1 result. All whole-blood, serum, and plasma specimens were tested by ICT-1. In order to assess the impact of freezing and thawing of the serum samples on the test results, three fresh specimens were put through 1, 5, and 10 freeze-thaw cycles and then retested by ICT-1. At approximately 3 months after they had been frozen, the sera were retested by ICT-1 to assess whether the sera had deteriorated during freezing and were concomitantly tested by ICT-2 to assess whether the manufacturer's modifications of the ICT Tuberculosis test improved its performance. For ICT-2, 35 μl of serum was tested after the serum was thawed. The results were recorded and interpreted as described above.

All data were entered into a database by the investigators. Analyses were carried out with Stata Statistical Software (release 6.0, 1999; Stata Corporation, College Station, Tex.) and Epi Info (version 6.04b, 1997; Centers for Disease Control and Prevention, Atlanta, Ga.). The sensitivity and specificity of tests with each antigen were assessed separately, as were the overall sensitivities and specificities of ICT-1 and ICT-2. Yates' corrected P values were used for comparisons of the sensitivities and the specificities. Results were stratified by TST (skin test positivity), history of vaccination with BCG, history of previous TB, smear results for AFB, HIV infection, and AIDS.

RESULTS

Patient population. The race, sex, age, and HIV serological status of the 70 patients suspected of having PTB and 42 controls are shown in Table 1. The group A controls were significantly less likely to be male than the group B controls ($P = 0.01$) or patients suspected of having PTB ($P < 0.001$). The group B controls were significantly younger than the group A controls ($P < 0.001$) or patients suspected of having PTB ($P < 0.001$).

Among the 70 patients suspected of having PTB, mycobacteria were identified by culture in 20 individuals (patients with PTB). In 15 of these 20 patients, *M. tuberculosis* species identification was made on the basis of the results of biochemical tests. Biochemical identification as mycobacterial species was not possible for the other five isolates due to insufficient growth, and resources were not available for nucleic acid probe testing. The five patients from whom the five isolates were obtained were considered to have PTB on the basis of their response to treatment directed against TB and on the basis of

TABLE 1. Characteristics of study subjects

Characteristic	Group A controls ($n = 21$)	Group B controls ($n = 21$)	Patients suspected of having PTB ($n = 70$)
Race (% of subjects)			
White	67	95	59
Nonwhite	33[a]	5	41
Sex			
Male	10[b]	43	69
Female	91	57	31
Median age (yr)	47	20[c]	46
HIV infection status			
Negative	100	100	73
Positive	0	0	24
Unknown	0	0	3

[a] $P = 0.05$ compared with the group B controls.

[b] $P = 0.01$ compared with the group B controls, and $P < 0.001$ compared with patients suspected of having PTB.

[c] $P < 0.001$ compared with the group A controls and patients suspected of having PTB.

TABLE 2. Specificities of ICT-1 and ICT-2 among controls and patients suspected of having PTB

Group	No. of subjects positive/no. of subjects tested (% [95% CI])			
	ICT-1, whole blood	ICT-1, plasma	ICT-1, serum	ICT-2, serum
Group A controls	20/21 (95 [76–100])	20/21 (95 [76–100])	21/21 (100 [87–100])	20/21 (95 [76–100])
Group B controls	15/21 (71 [48–89])	18/21 (86 [64–97])	18/21 (86 [64–97])	17/21 (81 [58–95])
TST positive (induration, ≥10 mm)	2/4 (50)	4/4 (100)	4/4 (100)	3/4 (75)
TST negative (induration, 5–9 mm)	3/3 (100)	2/3 (67)	2/3 (67)	2/3 (67)
TST negative (induration, 0–4 mm)	10/14 (71)	12/14 (86)	12/14 (86)	12/14 (86)
Vaccinated with BCG	13/19 (68)	16/19 (84)	16/19 (84)	15/19 (79)
Not vaccinated with BCG	2/2 (100)	2/2 (100)	2/2 (100)	2/2 (100)
Patients suspected of having PTB	18/39 (46 [30–63])	24/43 (56 [40–71])	28/42 (67 [51–80])	26/40 (65 [48–79])
Vaccinated with BCG	10/20 (50)	10/21 (48)	9/13 (69)	12/20 (60)
Not vaccinated with BCG	8/18 (44)	14/21 (67)	15/20 (75)	13/19 (68)
HIV positive	4/9 (44)	6/10 (60)	7/10 (70)	6/9 (67)
HIV positive without AIDS	2/5 (40)	2/5 (40)	3/5 (60)	3/5 (60)
HIV positive with AIDS	2/4 (50)	4/5 (80)	4/5 (80)	3/4 (75)
HIV negative	13/29 (45)	18/32 (56)	21/31 (68)	20/31 (65)

the previously established high positive predictive value (PPV) of an *M. tuberculosis*-positive culture of a respiratory specimen for this population (4). For 50 patients suspected of having PTB, mycobacteria were not identified by sputum culture. Among the 70 patients suspected of having PTB, sputum smears for AFB were 75% sensitive (95% confidence interval [CI], 51 to 91%) and 100% specific (95% CI, 93 to 100%) when culture of *M. tuberculosis* was considered the reference standard.

ICT-1. Six whole-blood specimens from patients suspected of having PTB gave invalid results by ICT-1 because the specimens clotted before they ran down the nitrocellulose strip. One serum specimen from a patient suspected of having PTB yielded invalid results because the serum was viscous and did not run down the nitrocellulose strip within 2 min in two attempts.

The specificities of ICT-1 for controls and patients suspected of having PTB are shown in Table 2. Among the controls, the specificities ranged from 71 to 100%, while among patients suspected of having PTB the specificities were poorer, ranging from 46 to 67%. Although for all specimen types the specific-

ities of ICT-1 were lower for the group B controls than for the group A controls, these differences were not statistically significant. In addition, the differences in the specificities for the group B controls with respect to TST status and vaccination with BCG were not significant. The specificities of ICT-1 for the different specimen types from patients suspected of having PTB were similar, and there were no significant differences in specificities for subgroups of patients of having PTB.

The sensitivities of ICT-1 are shown in Table 3. For all specimen types, the sensitivities of ICT-1 were higher for patients who were smear positive for AFB than for patients who were smear negative, but these differences were not statistically significant. The sensitivities of ICT-1 were not statistically different among patients with PTB vaccinated with BCG versus patients with PTB not vaccinated with BCG, patients with PTB who were HIV positive versus patients with PTB who were HIV negative, or patients with PTB who were HIV positive and who had AIDS versus patients with PTB who were HIV positive but who did not have AIDS. In addition, there were no differences in sensitivity among patients with PTB with a prior

TABLE 3. Sensitivities of ICT-1 and ICT-2 among patients with PTB

Patient group or characteristic	No. of patients positive/no. of patients tested (% [95% CI])			
	ICT-1, whole blood	ICT-1, plasma	ICT-1, serum	ICT-2, serum
All patients	15/18 (83 [59–96])	14/20 (70 [46–88])	13/20 (65 [41–85])	14/20 (70 [46–88])
Smear positive for AFB	13/13 (100)	13/15 (87)	12/15 (80)	12/15 (80)
Smear negative for AFB	2/5 (40)	1/5 (20)	1/5 (20)	2/5 (40)
Vaccinated with BCG	9/12 (75)	10/14 (71)	13/21 (62)	12/14 (86)
Not vaccinated with BCG	6/6 (100)	4/6 (67)	3/6 (50)	2/6 (33)
HIV positive	4/5 (80)	2/5 (40)	2/5 (40)	4/5 (80)
Without AIDS	3/4 (75)	2/4 (50)	2/4 (50)	4/4 (100)
With AIDS	1/1 (100)	0/1 (0)	0/1 (0)	0/1 (0)
HIV negative	10/12 (83)	11/14 (79)	10/14 (71)	9/14 (64)

TABLE 4. PPVs and NPVs of ICT-1 and ICT-2 for
patients suspected of having PTB

Prevalence (%) of patients culture positive for TB	ICT-1, whole blood		ICT-1, plasma		ICT-1, serum		ICT-2, serum	
	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)	PPV (%)	NPV (%)
10	15	96	14	94	18	95	18	95
29[a]	39	87	39	82	45	82	50	84
50	61	73	60	65	66	66	67	68

[a] Observed prevalence.

history of TB versus patients with PTB without a prior history of TB (data not shown).

The PPVs and negative predictive values (NPVs) of ICT-1 for patients suspected of having PTB were calculated for prevalences of PTB of 10, 29 (observed), and 50% among patients suspected of having PTB (Table 4). For the observed data, the PPVs were ≤50% and the NPVs were 82 to 87%.

Among the group B controls, patients with PTB, and patients suspected of having PTB but found not to have PTB, there were no significant differences in the distributions of positive test bands, the intensities of positive band signals, and the numbers of positive bands (data not shown). Test bands 3 and 4 were the most commonly positive bands for these groups for all specimen types. Among the group A controls, only one individual had a positive ICT-1 result for whole blood and plasma, and this was a single positive band (band 1) for each of these two specimen types. However, one patient with PTB also had a single positive band 1 (for all three specimen types).

For three serum specimens tested, multiple freeze-thaw cycles did not affect the ICT-1 results (data not shown). After 3 months of frozen storage, all serum samples were retested by ICT-1, and the calculated sensitivities and specificities from the first to the second round of testing were similar (data not shown).

ICT-2. A total of 102 frozen serum specimens were tested by ICT-2, of which 60 were from patients suspected of having PTB, 21 were from the group A controls, and 21 were from the group B controls. There were no invalid ICT-2 results for the frozen sera.

The specificities of ICT-2 for controls and patients suspected of having PTB are shown in Table 2. Among the group B controls, the specificity of ICT-2 was not significantly different for TST-positive versus TST-negative patients or for patients with a history of vaccination with BCG versus those without a history of vaccination with BCG. For patients suspected of having PTB, the specificities of ICT-2 were not different among the subgroups indicated in Table 2.

The sensitivities of ICT-2 for patients with PTB are shown in Table 3. The sensitivity of ICT-2 was higher for patients with PTB whose sputum was smear positive for AFB than for patients with PTB whose sputum was smear negative for AFB, but the difference was not statistically significant. The sensitivities of ICT-2 were not significantly different for the subgroups of patients with PTB indicated in Table 3. The PPVs and NPVs of IVT-2 are shown in Table 4. Overall, the manufacturer's modifications to ICT-1 did not substantially improve its performance.

For the group B controls, patients with PTB, and patients suspected of having PTB but found not to have PTB, there were no significant differences in the distributions of positive test bands, the intensities of positive band signals, and the numbers of positive bands (data not shown).

DISCUSSION

This study evaluated the sensitivities, specificities, PPVs, and NPVs of ICT-1 and ICT-2 for the detection of active PTB among patients suspected of having PTB and healthy controls in Rio de Janeiro City, Brazil. The performance of ICT-1 was evaluated with whole-blood, plasma, and serum specimens; and the performance of ICT-2 was evaluated with serum. Among patients suspected of having PTB, test sensitivities ranged from 65 to 83%, with specificities ranging from 46 to 67%. Among healthy controls who were either TST positive or vaccinated with BCG, specificities ranged from 71 to 86%, and among healthy controls who were TST negative and not vaccinated with BCG, specificities ranged from 95 to 100%. There were no significant differences in the sensitivities or the specificities of ICT-1 with whole blood, plasma, or serum. Among the patients suspected of having PTB and the controls, the specificity of ICT-2 with serum was slightly but not significantly lower than that of ICT-1. Among study subjects with a positive ICT-1 or ICT-2 result, neither the pattern of positive test bands nor the total number of positive bands distinguished persons with PTB from persons without PTB.

The ICT Tuberculosis tests are designed to detect IgG antibodies to five antigens secreted by *M. tuberculosis* in a technically simple and inexpensive card format. Since antibody responses to at least some *M. tuberculosis* antigens may be heterogeneous from person to person (12), the use of multiple antigens would be predicted to increase the sensitivity of the test. In our study, the sensitivities of ICT-1 and ICT-2 were similar or slightly higher than those reported previously (3, 14). However, the predictive value of a positive test result (PPV) or a negative test result (NPV) was too low to be clinically useful for our study population.

In this study, the sensitivities of ICT-1 and ICT-2 were higher for patients with PTB whose sputum was smear positive for AFB than for patients with PTB whose sputum was smear negative for AFB, although the number of patients with PTB whose sputum was smear negative for AFB included in the study was small and the differences were not statistically significant. This trend has been observed in other studies of antibody-based serodiagnostic tests for TB (1, 2, 15, 16). Whether this simply reflects a quantitatively greater antibody response in patients whose sputum is smear positive for AFB and has a high bacillary burden (11, 17) or a qualitatively different immunological response to *M. tuberculosis* is not clear and warrants further study. The low sensitivity of the ICT Tuberculosis tests for patients with PTB included in the study whose sputum was smear negative for AFB is disappointing, because this group of patients would potentially derive benefit from a rapid diagnostic blood test. However, since the overall PPVs of the ICT Tuberculosis tests with serum were calculated to be 66 to 67% in settings with a very high prevalence of PTB (>50% prevalence of PTB among patients suspected of having PTB), further evaluation of the ICT Tuberculosis tests with serum

from larger numbers of patients whose sputum is smear negative for AFB may be warranted in order to more definitively assess the tests' utility in settings with a very high prevalence of TB but without a laboratory able to perform mycobacterial culture.

The calculated high NPVs (94 to 96%) of the ICT Tuberculosis tests in a setting with a very low prevalence of PTB (a <10% observed prevalence of PTB among patients suspected of having PTB) raises the possibility that the ICT Tuberculosis tests may have some utility in excluding the diagnosis of PTB in such settings.

A major strength of this study is the evaluation under field conditions of the specificities of ICT-1 and ICT-2 for PTB in a population of patients who were suspected of having PTB and who had symptoms and/or clinical signs compatible with PTB. In this population, in which the prevalence of PTB among patients suspected of having PTB was 29%, the low specificities of ICT-1 and ICT-2 for PTB and the relatively low PPVs and NPVs limited the usefulness of the tests. An additional strength of this study is the evaluation of the patterns of positive test bands and the total number of positive test bands for the study subjects. Although neither of these was found to be useful in distinguishing subjects with PTB from those without PTB, to the best of our knowledge this type of analysis has not been performed previously for the ICT Tuberculosis tests.

A potential limitation of this study is that *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 ICT Tuberculosis tests. However, this is unlikely since none of the patients who were suspected of having PTB but who were assigned to the group considered not to have PTB were reported as having confirmed TB to the TB surveillance system of the state of Rio de Janeiro during the year following the conclusion of the study. In addition, relatively few cases of PTB were identified in this prospective study. This limited the subgroup analysis and contributed to wide 95% CIs. Finally, there was a significant age difference between the group B controls and both the group A controls and patients suspected of having PTB. This age difference reflected the initiation of mandatory vaccination of all Brazilian infants with BCG in 1976. While this age difference is not optimal, endemic controls were used because the inclusion of nonendemic controls would have severely confounded the results.

In conclusion, neither ICT-1 nor ICT-2 was sufficiently predictive for use as a routine diagnostic aid for PTB in a setting with a 29% observed prevalence of PTB among patients suspected of having PTB. However, further evaluation of the performance of the ICT Tuberculosis tests in selected settings may be warranted. A better understanding of the repertoire and dynamics of antibody responses in patients with *M. tuberculosis* infection and other mycobacterial infections may facilitate the development of more sensitive and specific antibody-based methods for the diagnosis of active PTB disease.

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