# Evaluation of a rapid immunochromatographic test for the serologic diagnosis of tuberculosis in Italy

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**Objective** To determine specificity, sensitivity and predictive values of a rapid immunochromatographic assay (ICT tuberculosis) for the diagnosis of tuberculosis (TB) in an Italian clinical setting, and to identify tentative new guidance for the interpretation of test results.

**Methods** The ICT tuberculosis test is an immunochromatographic test based on the detection of IgG antibodies directed against five highly purified antigens secreted by *Mycobacterium tuberculosis* during active growth. Sera from 60 patients with active pulmonary (48 sputum smear-positive and six sputum smear-negative cases) and extrapulmonary (six cases) TB were obtained. Personal, anamnestic and clinical data were investigated and recorded for each patient. The control groups comprised 156 subjects: 40 healthy individuals, half of them *Mycobacterium bovis* BCG-vaccinated, and 116 patients with mycobacterial diseases other than TB (five cases), with nonmycobacterial lung diseases (30 cases), with nonmycobacterial nonlung diseases (30 cases), with nonmycobacterial diseases and rheumatoid factors positivity (30 cases), and with asymptomatic HIV infection (21 cases). For 21 individuals the test was simultaneously performed with both serum and whole blood sample. Each positive result of the ICT test was reported with regard to the number (1–4), position (A, B, C, D) and color intensity (+ to ++++) of the evidenced lines in order to assess the quality of the antibody response.

**Results** The overall sensitivity and specificity were 56.7% and 90.4%, respectively. The sensitivity for pulmonary TB patients was 61.1% (66.7% for smear-positive and 16.7% for smear-negative cases) and 16.7% for extrapulmonary TB patients. The difference between ICT results in pulmonary TB patients and control subjects was statistically significant ($P < 0.0001$). The analysis of the positive ICT tests revealed that samples with strong color intensity ($\geq$++) and specific antibodies bound to antigens immobilized on line D were significantly more frequent in TB patients than in controls ($P = 0.001$ and $P = 0.027$, respectively). ICT test results with the presence of at least three visible lines were more often observed in the TB patients than in controls, although not reaching statistical significance ($P = 0.052$). No difference was observed between the results of the ICT test performed both on serum and whole blood sample.

**Conclusions** The ICT tuberculosis test was confirmed to be rapid and easy to perform without requiring special equipment, both on serum and whole blood sample. Our data, in accordance with those obtained in a previous study conducted in extra-European countries, confirmed higher sensitivities for the smear-positive TB patients than for the smear-negative TB patients, and for pulmonary TB patients than for the extrapulmonary

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TB patients. Data obtained on the quality of antibody response in the ICT positive samples, might be used to improve the performance of the test.

**Keywords** tuberculosis, serodiagnosis, ICT tuberculosis, immunochromatographic assay

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## INTRODUCTION

The tuberculosis (TB) epidemic is growing larger and more dangerous each year. In 1999 there were an estimated 8.4 million new TB cases, while there were up to 8.0 million in 1997 [1]. The rise is due largely to a 20% increase in incidence in the African countries most affected by the epidemic of HIV/AIDS. If the present trend continues, 10.2 million new cases are expected in 2005. On a global scale, TB has a devastating impact on developing countries, with 23 countries accounting for nearly 80% of all new cases [1]. Control strategies are based on the rapid and accurate detection of infected individuals and appropriate chemotherapy.

The diagnosis of TB in developing countries relies largely on microscopic detection of acid-fast bacilli in smears from clinical specimens. However, this low cost and rapid method is characterized by a high variable sensitivity (22–80%), particularly low for paucibacillary and extrapulmonary TB patients [2].

Culture methods are more sensitive and specific but are time consuming, take several weeks to become positive and are not available in many peripheral diagnostic services.

The new rapid diagnostic tests based on molecular techniques, such as polymerase chain reaction or nucleic probes, are sensitive and specific. However, their use in developing countries is not practicable due to the high cost and the need of well-trained staff and sophisticated laboratory facilities [3].

An alternative to microscopy is needed for the diagnosis of TB in developing countries, but it must be rapid, simple, inexpensive and reliable. Serology is potentially useful but the diagnostic tests using enzyme-linked immunosorbent assay (ELISA) methodology which have been evaluated so far present a high variability in terms of sensitivity (16–100%) and specificity (71–100%) [4–7].

Recently, a rapid immunochromatographic assay, ICT tuberculosis (AMRAD ICT, Australia), which requires no special equipment and little technical skill, has been developed and commercialized for the serodiagnosis of TB. According to published experiences with patients from China, California (USA), New Zealand, Korea, and Madagascar, the sensitivity of the test varied from 20 to 92%, and specificity ranged from 83 to 96% [7–12].

We evaluated for the first time in a European setting the performance of the test with the aim of assessing sensitivity, specificity, and positive and negative predictive value, and of identifying tentative new guidance for test-result interpretation.

## MATERIALS AND METHODS

### Study population

Over 15 months, from October 1999 to December 2000, a total of 216 individuals were enrolled. The hospitals involved were the Careggi Hospital (Divisions of Infectious Diseases and Division of Respiratory Diseases) and the Santa Maria Annunziata Hospital (Division of Infectious Diseases), both located in Florence, Italy. Sixty were patients diagnosed as having active pulmonary or extrapulmonary TB, and 156 were selected as controls. The diagnosis of pulmonary TB was based on the results of sputum smear and culture. Patients with extrapulmonary TB were diagnosed by microbiological or histological results of specimens from the site of infection.

Personal and anamnestic data, when available, were recorded for each patient reporting smoking behavior, BCG vaccination status, HIV infection, prior clinical history of TB, and date of serum collection. The current TB illness was investigated for time of disease onset, infection site, coinfection initiation, and treatment date. Moreover, the results of specimen smear and culture, X-ray with number and size of possible cavities, ligase chain reaction (LCR) for *M. tuberculosis* complex on different samples (sputum, blood, urine, abdominal fluid), and Mantoux test were reported. The Centers for Disease Control and Prevention interpretative criteria for positive Mantoux test results were followed [13].

TB patients ($n = 60$) selected for this study had a median age of 38 years (age range, 18–90 years),

and 39 of them (65%) were males. Forty-six patients were Caucasian, seven African, six Asiatic and one Latin-American. Twenty out of 42 patients reported as being smokers, 11 of 17 patients had a prior clinical history of TB, usually more than ten years before, only two patients were BCG vaccinated, and none had antibodies to HIV.

With regard to the current TB illness, 54 (90%) were pulmonary cases and six (10%) were extrapulmonary cases. Among the patients with pulmonary TB, 48 (88.9%) were smear- and culture-positive and six (11.1%) were smear-negative and culture-positive. The species isolated was in all cases *M. tuberculosis*. All the patients were screened by radiograph: 28 patients presented evidence of cavities and half of them had more than one cavity. The size of the cavities ranged between 15 and 50 mm. Patients with extrapulmonary TB were divided according to the location of the affected organ in lymphonodal TB (two patients), genitourinary TB (two patients), peritoneal TB (one patient) and bone TB (one patient). Twenty-four patients were screened by the Mantoux test, and 18 had a positive result ranging from 10 to 30 mm. In 25 out of 32 patients the diagnosis was confirmed by LCR carried out on sputum (27 cases), urine (one case), gastric fluid (one case), lymph caseosis (one case) and other specimens (two cases). Seven patients had coinfections: six with chronic hepatitis and one with streptococcal tonsillitis. All patients diagnosed as having TB received anti-TB chemotherapy. The distance between initiation of therapy and serum collection ranged from 0 to 213 days, with a median value of 6.5 days, and between onset of disease and serum collection from two to 390 days, with a median of 45 days.

The control group comprised 40 healthy individuals, half of them vaccinated with *M. bovis* BCG vaccine, and 116 non-TB patients (five with non-TB mycobacterial infections, 30 with nonmycobacterial lung diseases, 30 with nonmycobacterial non-lung diseases, 30 with nonmycobacterial diseases associated with positivity for rheumatoid factors, and 21 asymptomatic HIV patients with CD4 count $> 200$/mL). Patients with non-TB mycobacterial infections were diagnosed in the presence of clinical and/or radiological evidence and positive culture on at least two occasions. Patients with non-TB diseases were diagnosed according to the standard clinical practice for each disease.

The 156 subjects selected for the control group had a median age of 44.5 years (age range, 14–88 years) and 88 (56.4%) were males. The differences of age and sex between the TB patients and control groups were not statistically relevant.

After obtaining informed consent, a sample of peripheral blood was taken from each subject and the serum was stored at $-70$ °C. All the sera of the study patients were tested for antibodies to HIV by ELISA (Vironostika HIV, Uniform II Plus-O, Organon Teknika, Holland).

### Principle of test

The ICT tuberculosis test is an immunochromatographic test based on the detection of IgG antibodies directed against five highly purified antigens, including one of 38 kDa, secreted by *M. tuberculosis* during active infection. The details of the test have been reported elsewhere [9]. Briefly, the test consists of a cardboard folding device containing a nitrocellulose strip on which antigens are immobilized on four lines. When a sample (serum, plasma or whole blood) is applied, it flows past these antigens and, if specific antibodies to these antigens are present, they bind to one or more of the lines. Bound antibodies are detected by a goat antihuman IgG conjugated to colloidal gold particles that give a pink line when bound to human antibody. The test is completed in 15 min and is considered positive when at least one of the four antigen lines and the control line develop a pink color.

Testing was performed strictly according to the instructions outlined in the product insert. For 21 individuals the test was simultaneously performed with serum sample and with whole blood sample in order to compare the results. All test results were read separately by two operators unacquainted with the patient diagnosis. Each positive result was recorded with regard to the number, position and intensity of the pink lines. For the identification of positions, the lines were named A, B, C, and D where A indicated the position next to the control line.

### The color intensities were classified into the following categories

+ (weak positive), a faint pinkish red line;
++ (positive), an easily visible pinkish red signal, lighter than the control line;
+++ (strong positive), a pronounced reddish signal, similar to the control line;

++++ (very strong positive), a deep purple-red signal, darker than the control line.

When an operator was not able to definitely categorize a signal into one of the categories listed above, the test was repeated.

### Statistical analysis

Data were analyzed using the Epi Info 6 statistical package [14]. Sensitivities, specificities, and predictive values were calculated by standard methods [15]. Sensitivity was defined as the ability to detect cases of active TB. Specificity was defined as the ability to be negative for the control groups, who were considered to be free of active TB. Positive predictive value (PPV) was defined as the probability that a subject with a positive test actually had active TB, and negative predictive value (NPV) as the probability that a subject truly did not have active TB, given a negative test. Statistical significance of differences in frequency was measured using the $\chi^2$ test, and Fisher's exact test when appropriate. Positive and negative predictive values were calculated for different prevalences of disease: the prevalence of TB among respiratory patients being admitted to our infectious diseases ward (22.5%), and the overall prevalence rate of TB (0.0031%) in Italy [16].

### Results

The ICT test detected 33 out of 54 (61.1%) pulmonary TB patients and one of six (16.7%) extrapulmonary patients. The overall sensitivity of the assay was 56.7% (34 out of 60 patients) (Table 1). The difference between ICT results in pulmonary TB patients and control subjects was statistically significant ($P < 0.0001$). No statistically significant difference was found between ICT results in extrapulmonary TB patients and controls ($P = 0.39$).

When the number of patients in the pulmonary TB category was divided on the basis of sputum smear and culture results, the assay detected 32 out of 48 (66.7%) TB patients in the sputum smear- and culture-positive groups and one out six (16.7%) in the sputum smear-negative and culture-positive groups. The antibody detection rate in the sputum smear-positive group was significantly higher ($P = 0.028$).

The differences of ICT positivity in the categories of patients reporting a previous history of clinical disease ($P = 0.34$), smoking ($P = 0.18$), coinfections ($P = 0.43$), or positive results for LCR ($P = 0.15$) were not statistically significant when compared to patients negative for these factors. No relationship was apparent between positive ICT results and the ethnic origin of patients (Caucasian vs. non-Caucasian) ($P = 0.23$), positive Mantoux test ($P = 0.78$), presence of X-ray cavities ($P = 0.10$), and length of disease (>60 days) >60 days) ($P = 0.39$) or anti-TB chemotherapy (>60 days) ($P = 0.21$) before serum collection.

Fifteen out of 156 control subjects tested positive with the ICT test giving a specificity of 90.4%. The test specificity for the different control groups is

**Table 1** ICT Tuberculosis test results on tuberculosis (TB) patients and control subjects

| | Number of subjects (%) | Number positive | Sensibility (%) | Specificity (%) |
|---|---|---|---|---|
| TB patients | | | | |
| Pulmonary TB | | | | |
| smear-positive | 48 (80) | 32 | 66.7 | – |
| smear-negative – culture-positive | 6 (10) | 1 | 16.7 | – |
| **Total pulmonary TB** | **54 (90)** | **33** | **61.1** | **–** |
| Extrapulmonary TB | 6 (10) | 1 | 16.7 | – |
| **Total TB patients** | **60 (100)** | **34** | **56.7** | **–** |
| Controls | | | | |
| Nontubercular mycobacterial infections | 5 (3.2) | 0 | – | 100 |
| Nonmycobacterial lung diseases | 30 (19.2) | 4 | – | 86.7 |
| Nonmycobacterial nonlung diseases | 30 (19.2) | 3 | – | 90 |
| Nonmycobacterial diseases with rheumatoid factors positivity | 30 (19.2) | 3 | – | 90 |
| Asymptomatic HIV patients with CD4 > 200/mL | 21 (13.5) | 1 | – | 95.2 |
| Healthy individuals BCG vaccinated | 20 (12.8) | 0 | – | 100 |
| Healthy individuals | 20 (12.8) | 4 | – | 80 |
| **Total control subjects** | **156 (100)** | **15** | **–** | **90.4** |

showed in Table 1. Statistically, the differences between these groups were not significant. None of the five patients with non-TB mycobacterial infection and none of the 20 healthy subjects with previous BCG vaccination had a false-positive response.

Analyzing the quality of the antibody response in ICT-positive samples, the lines A, B, C, and D were visible in 32.4%, 58.8%, 47.1%, and 58.8% of the 34 TB patients, and in 26.7%, 46.7%, 40%, and 20% of the 15 control subjects, respectively. Only the presence of line D was significantly more frequent in TB patients than in controls ($P = 0.027$).

In Figure 1 we present the result relative to the distribution of the number of visible lines in the ICT tuberculosis-positive subjects. The presence of at least three visible lines was much more frequent in TB patients (11/34) than in controls (1/15), although not reaching statistical significance ($P = 0.052$).

With regard to the intensity of the positive signals, the +, ++, +++, and ++++ categories were observed in 37.3%, 38.8%, 13.4%, and 10.4% of the 67 lines visible in TB patients. In the control group, 90% and 10% of the 20 visible lines were classified into + and ++ categories, respectively. No false-positive ICT test presented a color intensity classifiable into categories +++ and ++++ (Figure 2). Positive tests with signals equal to or stronger than ++ had statistically significant association with TB cases ($P = 0.001$).

No difference was observed between the results of the test performed both on serum and whole blood samples from 21 subjects (15 TB cases and six controls).

ICT assay proved to be easy to perform and interpret. No discrepancy between the two operators was observed except for few cases of minor discordance in the classification of intensity of the lines.

According to the Bayes' theorem, PPV and NPV of the test used with patients admitted to one of the wards of infectious diseases, where the study was carried out and where the prevalence of TB among respiratory patients is 22.5%, were 64.8% and 88.8%, respectively. In contrast, PPV and NPV, calculated according to the prevalence of TB in Italy (0.0031%), were 0.00019% and 99.9%, respectively.

## DISCUSSION

Despite recent advances in identifying and purifying antigens secreted in active tuberculosis infection with diagnostic potential for detecting specific antibodies from TB patients, the serological assays currently used still present substantial limitations and have not acquired a defined role in clinical practice. We have evaluated in our setting a single and rapid immunochromatographic assay, ICT tuberculosis, already tested in extra-European countries with variable results. A previous version

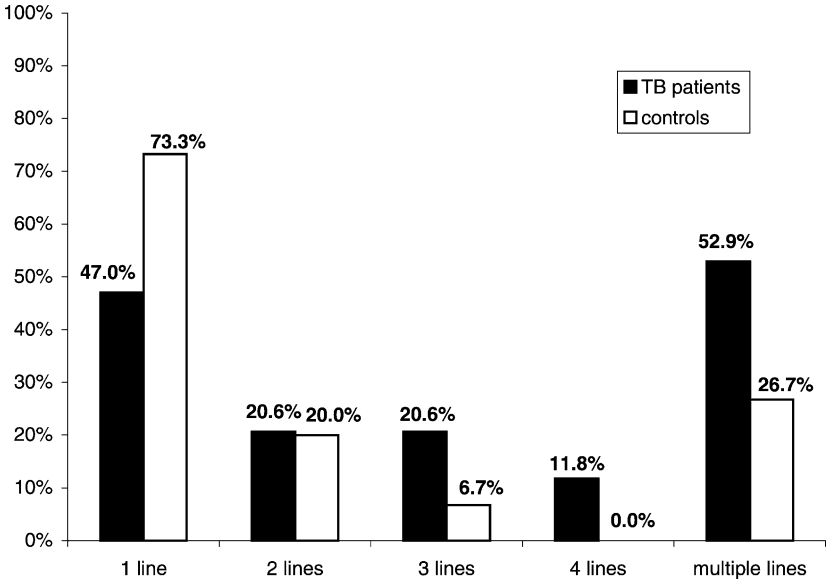


**Figure 1** Distribution of positive ICT tuberculosis test according to the number of visible lines.

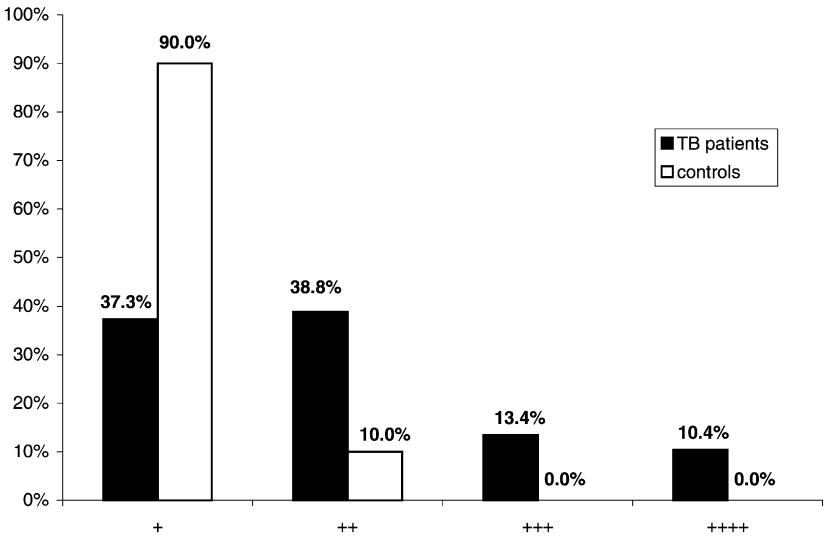


**Figure 2** Distribution of positive ICT tuberculosis test according to the color in intensity of the visible lines.

of ICT tuberculosis (which contained only the 38 kDa antigen), tested in China on 152 pulmonary TB patients, showed a sensitivity of 89% for smear-positive patients and a sensitivity of 74% for smear-negative patients, with a specificity of 93% [9]. In another study carried out in China on 268 TB patients, the same test showed a sensitivity of 92% and 70% in smear-positive and smear-negative pulmonary TB patients respectively, and 76% for extrapulmonary TB patients, with an overall specificity of 92% [12].

The new version of ICT tuberculosis, which utilizes five highly purified antigens (including 38 kDa) secreted by *M. tuberculosis*, showed a sensitivity of 48% and 18% in pulmonary and extrapulmonary TB patients respectively, in a study conducted in New Zealand on 44 patients with active TB, 33 of whom had pulmonary disease [7]. Specificities of 96% and 88% were found with sera from control patients who had undergone the Mantoux test and anonymous controls, respectively. In a study carried out in Madagascar, ICT tuberculosis had a sensitivity of 68.2% in the diagnosis of 22 smear-positive pulmonary TB cases, and of 65.2% for 23 extrapulmonary TB patients. The specificity was 83.3% [11]. A sensitivity of 73% was found in a study carried out in Korea on 37 TB patients, of whom 33 had pulmonary TB. In the same study specificities of 88%, 94%, and 94% were found in healthy adults, hospital workers, and non-TB patients, respectively [8].

A lower sensitivity (20%) was found in a study carried out in California, USA, on 59 culture-positive pulmonary and extrapulmonary TB patients, including HIV-positive individuals. The sensitivity increased to 31% when calculated by considering only the 52 HIV-negative subjects and to 46% when limited to HIV-negative cases with more than three months duration of disease. The overall specificity was 89% [10].

In our study, the first carried out in Europe, ICT tuberculosis achieved an overall moderate sensitivity (56.7%) with values ranging from 66.7% in smear-positive pulmonary TB patients to 16.7% in smear-negative and culture-positive pulmonary TB patients, and 16.7% in extrapulmonary TB patients. Our data, in accordance with those obtained by other investigators, confirmed higher sensitivities for the smear-positive patients than for the smear-negative patients, and for the pulmonary-infection patients than for the extrapulmonary-infection patients. The more vigorous antibody response observed in smear-positive patients has been interpreted as a result of a greater exposure to antigens in patients with high bacillary loads [7,17]. However, it is important to note that the sensitivity of ICT tuberculosis (61.1%) in our pulmonary TB patients is significantly less than that (88.9%) of the microscopic detection of acid-fast bacilli in smears ($P < 0.01$). The low sensitivity (16.7%) we observed in extrapulmonary TB patients is similar to that (18%) found in the study carried out in New Zealand [7], but differs from those reported from China (76%) [12] and Madagascar (65.2%) [11].

An increase in antibody levels has been associated with more extensive TB [18], and with the duration of disease [12] or anti-TB chemotherapy

[12,19,20]. These factors were analyzed in our study but no significant associations were found.

As far as false-positive results are concerned, the overall specificity was 90.4%, confirming the good results obtained in previous studies. In particular, we did not find any false-positive results either in patients with non-TB mycobacterial infection, or in healthy subjects with previous BCG vaccination.

One of the aims of our study was to identify tentative new guidance for the interpretation of results. Analyzing the quality of the antibody response in ICT-positive samples, we observed that the presence of one of the four antigen lines (line D) was significantly more frequent in TB patients than in controls. We do not know the nature of the antigens applied to this line because the information is not divulged by the manufacturer, but they resulted in being more specific than the others. Considering the number of positive lines, we found four visible lines only in TB patients even if in a small percentage (12%) of them, and the presence of at least three visible lines much more frequently in TB patients than in controls, although this difference did not reach statistical significance.

The analysis of the intensity of the positive lines showed that strong positive signals (+++ or ++++) were present only in TB patients and that signals equal to or stronger than ++ were statistically associated with TB cases.

These findings might represent the basis improving the interpretation of result and the specificity of the test. Limiting the false-positive result is considered a primary objective in the development of a TB serologic test in order to avoid unnecessary anti-TB chemotherapy and to permit appropriate treatment of another undiagnosed condition [6].

The performance of a clinical test may differ if used in one setting or another, with predictive values depending on the frequency of disease in the group of patients being studied [15]. In our study, ICT tuberculosis test, performed and interpreted accordingly to the manufacturer's instructions, resulted in providing good negative predictive values, 99.9% and 88.8%, respectively, for exclusion of TB in low (0.0031%) and relatively high (22.5%) TB-prevalence populations. A positive test resulted in having a moderate positive predictive value (64.8%) to strengthen a clinical suspicion of TB in selected symptomatic respiratory patients, while having an unacceptably low value (0.00019%) when applied to a low TB prevalence population, such as the case of Italy.

In conclusion, the ICT tuberculosis test proved to be rapid and easy to perform without requiring special equipment, both on serum and whole blood sample. Predictive values of the test vary accordingly to the context of its use. However, the sensitivity and specificity demonstrated by the test do not suggest, for the moment, that the test should replace the standard methods for the diagnosis of tuberculosis.

Data obtained in this study on the quality of the antibody response in the ICT-positive samples may be helpful to users for interpretation of result as well as developing the manufacturer guidelines for interpreting guidance.

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