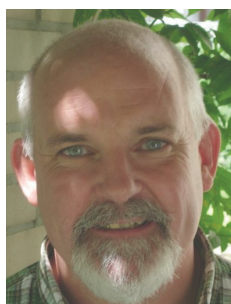


Commercial serological tests for the diagnosis of tuberculosis: do they work?



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Imperative to improve tuberculosis diagnostic tests

Worldwide, tuberculosis (TB) takes an enormous toll in morbidity and mortality. In 2005 alone, an estimated 8.8 million people developed TB and 1.6 million people died [1]. TB most commonly affects the lungs, but may involve any organ (extrapulmonary TB). In countries with comprehensive systems for diagnosis and reporting, extrapulmonary disease accounts for approximately 15–40% of reported cases [101,102]. In the USA in 2005, approximately 71% of TB cases were pulmonary disease and 20% were extrapulmonary disease. An additional 9% of cases had both pulmonary and extrapulmonary involvement [2].

Both pulmonary and extrapulmonary TB present diagnostic challenges. For the diagnosis of pulmonary TB, direct sputum-smear microscopy is often the sole diagnostic test used in low-income countries where the vast majority of patients reside. Microscopy has modest and variable sensitivity (22–80%) compared with culture methods [3,4]. In addition, microscopy contributes little to the diagnosis of pediatric pulmonary TB [5] and does not, by definition, identify smear-negative pulmonary TB, which is disproportionately higher in HIV-positive than HIV-negative individuals [6]. A major shortcoming of microscopy, as currently recommended, is the requirement for collection and microscopic examination of multiple sputum specimens [7], an issue that may result in delays in diagnosis [8].

Extrapulmonary TB occurs with greater relative frequency in persons with HIV infection and in children, than in adults [5,9]. The diagnosis of extrapulmonary TB is often difficult to establish, especially for patients in resource-limited areas. Signs and symptoms are nonspecific and microscopic examination for acid-fast bacilli lacks sensitivity for extrapulmonary disease [9]. Mycobacterial culture and histopathology are

more sensitive methods but are not commonly available. Invasive procedures that are complex and costly may be required to obtain the necessary diagnostic specimens [9]. As a result of these difficulties, misdiagnosis of extrapulmonary TB is common in all countries and may result in unnecessary treatment if falsely diagnosed, or greater morbidity and mortality if the diagnosis is missed, especially in persons with HIV infection [10,11].

The emergence of multidrug-resistant TB and now extensively drug-resistant TB has highlighted the importance of developing new, rapid and accurate TB diagnostics. In this article we assess the performance of commercial serological antibody detection tests for the diagnosis of pulmonary and extrapulmonary TB.

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Serological tests for the diagnosis of TB

Serological (i.e., humoral and antibody-mediated) tests are widely available, although no guideline recommends their use for the diagnosis of TB. By contrast, blood-based tests for cellular immune responses (i.e., T-cell-based IFN- γ release assays) have been recommended for use in certain countries [12]. Currently, in low-income countries, where diagnostic tests are rarely subjected to regulatory review or approval [13], test manufacturers and distributors are marketing dozens of different antibody detection commercial kits [103]. The extent of their use is largely unknown; however, companies report sales volumes between 3000 and 300,000 tests per year [14].

Serological tests would appear to offer the potential to improve TB diagnosis as currently performed, as some of the test formats are suitable for resource-limited areas. Major advantages of these tests are their speed (in some cases results may be available within minutes because of the

point-of-care format) and potential simplicity compared with microscopy. The ELISA format and immunochromatographic test format are two commonly used designs for serological tests. ELISA is a complex assay with several steps and takes several hours to perform. The immunochromatographic test is simpler and can be completed in a few minutes.

As part of a project commissioned by the WHO Special Programme for Research and Training in Tropical Diseases, a series of systematic reviews was conducted to synthesize existing evidence and define a research agenda for key areas in TB diagnostics. A previous editorial described the findings from three reviews on sputum microscopy [15]. The current paper focuses on antibody detection tests for the diagnosis of TB; in particular, findings from two systematic reviews of commercial serological tests for the diagnosis of pulmonary [16] and extrapulmonary TB [17] are summarized in Table 1 and described below. In addition, related findings from a comprehensive review of rapid TB diagnostics by Dinnes and coworkers are also discussed [18].

'Serological tests would appear to offer the potential to improve TB diagnosis ... as some of the test formats are suitable for resource-limited areas.'

How accurate are commercial serological tests for the diagnosis of pulmonary TB?

The review on commercial serological tests for the diagnosis of pulmonary TB identified 68 eligible studies from 27 publications (Table 1) [16]. Nine commercial tests (three different tests used distinct assays for detection of IgG, IgM and/or IgA antibodies) were represented in the review. Of the total 68 studies, only 17 (25%) were considered to be of high quality. The review yielded important findings:

- Overall, commercial tests varied widely in performance (sensitivity of 10–90% and specificity of 47–100%);
- Accuracy was higher in smear-positive (41 studies) than smear-negative samples (27 studies);
- In studies of smear-positive patients (ten studies), Anda-TB IgG (Anda Biologicals, Strasbourg, France) by ELISA (the test most frequently evaluated in the publications reviewed) demonstrated limited sensitivity (63–85%) and inconsistent specificity (73–100%);

- In smear microscopy-negative patients (four studies), sensitivity estimates for Anda-TB IgG were low and variable (64, 71, 73 and 35%); corresponding specificity estimates were higher (93, 89, 91 and 88%);
- Specificity was higher in healthy participants than in patients for whom TB disease was initially suspected and subsequently ruled out (a more appropriate test population);
- There were insufficient data to determine the accuracy of most commercial tests in smear-negative patients, as well as their performance in children or persons with HIV infection. This was surprising given the importance of these patient groups.

Similarly, Dinnes and coworkers found that commercial serological tests for pulmonary TB did not perform well [18]. Their review, which only included studies with a 'cohort' or 'case series' type design, identified eight eligible studies. As a whole, commercial tests demonstrated modest performance (diagnostic odds ratio [DOR] of 7.30 [95% CI: 1.95–27.24]) with a pooled mean sensitivity of 88% and mean specificity of 50%. When only studies with at least two quality criteria were considered (seven studies), the DOR declined to 6.35 (95% CI: 0.59–67.98) and the pooled mean sensitivity decreased to 34%.

How accurate are commercial serological tests for the diagnosis of extrapulmonary TB?

The review on commercial serological tests for the diagnosis of extrapulmonary TB identified 21 eligible studies from nine publications (Table 1) [17]. Only those studies that based the diagnosis of extrapulmonary TB on bacteriologically confirmed TB or the presence of caseating granulomas in histopathological specimens were included. Five commercial tests (one test used distinct assays for the detection of IgG, IgM and IgA antibodies) were represented in the review:

- Commercial tests were highly variable with respect to sensitivity (0–100%) and specificity (59–100%) for all extrapulmonary sites combined;
- Anda-TB IgG (the test most frequently evaluated in the publications reviewed) demonstrated highly variable sensitivity (26–100%) and specificity (59–100%) for all extrapulmonary sites combined (ten studies);

- For all commercial tests combined, sensitivity estimates for both lymph node TB (four studies, 23–100%) and pleural TB (four studies, 26–59%) were poor and inconsistent;
- There were no data to determine the accuracy of the tests for the diagnosis of extrapulmonary TB in children or patients with HIV infection, the two groups for which the test would be most useful.

The review identified only one study that assessed the accuracy of a commercial test in patients with meningitis [19]. In this study

(56 culture-confirmed patients and 74 hospitalized patients without TB), the sensitivity of the test was poor at 48% (95% CI: 35–62%) and specificity was modest at 82% (95% CI: 72–90%).

Dinnes and coworkers also found that commercial serological tests performed poorly in the diagnosis of extrapulmonary TB [18]. The authors identified three eligible studies that evaluated commercial tests for pleural TB. Sensitivity values in all three studies were less than 50% and specificity values ranged from 60 to 92%. For three different studies evaluating commercial tests for the diagnosis of miscellaneous sites of

Table 1. Findings from systematic reviews of commercial serological antibody detection tests for the diagnosis of pulmonary and extrapulmonary tuberculosis.

Systematic review	Total number of studies in the review	Median sample size*	Tests evaluated	Principal findings	Ref.
Pulmonary TB	68	41(38–75)/ 45 (40–107)	Anda-TB Detect-TB ICT TB Kaolin agglutination test MycoDot Pathozyme-Myco Pathozyme-TB Complex Plus TB enzyme immunoassay TB glycolipid assay	Overall, tests varied widely in performance (sensitivity, 10–90% and specificity, 47–100%). Sensitivity was higher in smear-positive than smear-negative samples. In studies of smear-positive patients, Anda-TB IgG showed limited sensitivity (63–85%) and inconsistent specificity (73–100%). Specificity was higher in healthy volunteers than in patients for whom TB disease was initially suspected and subsequently ruled out. There were insufficient data to determine the accuracy of most commercial tests in smear-negative patients, as well as their performance in children or persons with HIV infection.	[16]
Extrapulmonary TB	21	35 (30–56)/ 48 (37–194)	Anda-TB ICT TB Pathozyme-Myco Pathozyme-TB Complex Plus SEVA TB	All tests provided highly variable estimates of sensitivity (0–100%) and specificity (59–100%) for all extrapulmonary sites combined. Anda-TB IgG showed highly variable sensitivity (26–100%) and specificity (59–100%) for all extrapulmonary sites combined. For all tests combined, sensitivity estimates for both lymph node TB (23–100%) and pleural TB (26–59%) were poor and inconsistent. There were no data to determine accuracy of the tests in children or patients with HIV infection.	[17]

*Participants with TB/without TB (interquartile range).
TB: Tuberculosis.

extrapulmonary TB, sensitivity values were also low and inconsistent (59, 11 and 72%), although specificity estimates tended to be higher (82–99%).

Conclusions & policy implication

Available evidence suggests that, at this point in time, published data on commercial serological antibody detection tests for both pulmonary and extrapulmonary TB produce inconsistent estimates of sensitivity and specificity. For pulmonary TB, there were insufficient data to determine the accuracy of most commercial tests in smear microscopy-negative patients and none of the assays performed well enough to replace microscopy. For extrapulmonary TB, it is particularly disappointing that there were no studies of commercial tests of sufficient quality to enable their evaluation in patients with HIV infection, or in children, as it is in these groups that the tests could be most useful. Thus, these tests must be considered to have little or no role in the diagnosis of TB at the present time.

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Future perspective & research directions

Available evidence underscores the need for greater regulatory oversight of *in vitro* diagnostics and improved capacity in countries to design, conduct and report diagnostic test evaluations, which, in turn, can guide procurement and clinical practice. It is important that the literature from research laboratories that have evaluated the immunodiagnostic potential of different antigens be reviewed to determine whether there are useful antigens, which have been described but whose potential has not been fully exploited. Finally, it is essential that trials of new serodiagnostic tests for TB adequately address the particular challenges presented by smear microscopy-negative patients, children and people with HIV infection.

Over the past decades, much effort, as evidenced by the large number of published studies, has gone into developing a serodiagnostic test for TB based on antibody detection, but no test with adequate sensitivity and specificity has yet been identified. Why have these attempts failed?

Studies during the last decade have permitted an understanding of the lacunae of efforts so far and provided a basis for the research needed to develop an immunodiagnostic test for TB. A systematic analysis of the humoral immune responses produced by pulmonary TB patients has demonstrated that the profile of antigenic proteins of *Mycobacterium tuberculosis* recognized by antibodies differs at different stages of the natural history of infection and disease [20–22]. Thus, a serological test that can identify the different stages of clinical TB may need to be based on a combination of antigens. To the best of our knowledge, an analysis of antigens expressed during extrapulmonary replication of *M. tuberculosis* has not been performed. Identification and study of *M. tuberculosis* genes expressed in the different environments that characterize different sites of involvement may be able to provide the optimal reagents for devising a serodiagnostic test for extrapulmonary forms of the disease. It will also be important to examine proteins expressed by *M. tuberculosis* and the pattern of subsequent antigen recognition and antibody reactivity in HIV/TB-coinfected patients and children. Although much work needs to be done, the understanding of the humoral immune responses in TB patients and the new tools of genomics and proteomics may lead to the development of the simple and rapid serodiagnostic test that has eluded us so far.

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