Dear Working Group Members,

High quality diagnostic studies are critical to evaluate new tools, to develop evidence-based policies on TB diagnostics, and, ultimately, for effective control of the global TB epidemic. Systematic reviews and meta-analyses provide the best synthesis of current evidence and have the potential to impact policy. With the support of TDR/WHO and FIND, members of our Working Group (and other researchers) have conducted and published several meta-analyses and systematic reviews on various TB diagnostics (such as smear microscopy, serological tests, and NAAT). In addition to informing the field of evidence-based TB diagnosis, these reviews have been helpful in making policy decisions and in identifying key knowledge gaps and unresolved questions.

We are pleased to share with you a set of published systematic reviews on TB diagnostics, along with abstracts of these reviews. We believe this set of systematic reviews will be helpful to researchers, clinicians, and policy makers.

To continue work in this area of evidence-based TB diagnosis, we have created a new subgroup within our Core Working Group. This subgroup on Evidence Synthesis for TB diagnostics will be co-chaired by Madhukar Pai (McGill University, Montreal) and Richard O’Brien (Foundation for Innovative New Diagnostics [FIND], Geneva).

With very best regards

Dr Giorgio Roscigno, Chair.

Andrew Ramsay, Secretary.
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ADA: adenosine deaminase; IFN-γ: interferon-γ; INH: isoniazid; NAAT: nucleic acid amplification test; NA: not applicable; NR: not reported; PCR: polymerase chain reaction; RIF: rifampin; TB: tuberculosis
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Assessment by Meta-Analysis of PCR for Diagnosis of Smear-Negative Pulmonary Tuberculosis

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Received 8 August 2002/Accepted 23 March 2003

We conducted a meta-analysis to assess the performance of PCR for the diagnosis of smear-negative pulmonary tuberculosis (SPT) and to identify factors that account for differences in the diagnostic accuracy of different studies. Studies published before February 2002 were included if sensitivity and specificity of PCR in smear-negative respiratory or gastric-aspirate specimens could be calculated. Analysis was conducted by using summary receiver operating characteristics models. Sensitivity and specificity ranged from 9 to 100% and from 25 to 100%, respectively. Fewer than 40% of the 50 studies reported results by number of patients, reported clinical characteristics of patients, or used as a reference standard combined culture and clinical criteria. Studies that included bronchial specimens showed higher accuracy than studies that evaluated only sputum specimens or included gastric aspirates. Studies that did not report that tests were applied blindly showed higher accuracy than those reporting blind testing. Increased sensitivity due to the use of DNA purification methods was associated with decreased specificity. Studies published after 1995, using Amplicor or dUTP-UNG, were associated with an increase in specificity at the expense of lower sensitivity. We concluded that PCR is not consistently accurate enough to be routinely recommended for the diagnosis of SPT. However, PCR of bronchial specimens could be useful in highly suspicious SPT cases. Studies not reporting blind testing are likely to overestimate accuracy of PCR. Future evaluation of PCR accuracy should be conducted by patient and type of respiratory specimen, blindly, by using a reference standard that combines culture and clinical criteria and addresses the issue of how patient characteristics affect PCR accuracy.

Diagnostic value of adenosine deaminase in tuberculous pleural effusion: a meta-analysis

Masaehiro Goto, Yoshinori Noguchi, Hiroshi Koyama, Ken'iti Hira, Takuro Shimbo and Tsuguya Fukui

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Abstract
Background. Many studies have investigated the usefulness of adenosine deaminase activity (ADA) in pleural fluid for the early diagnosis of tuberculous pleurisy. To summarize the diagnostic characteristics of ADA we undertook a meta-analysis using a summary receiver operating characteristic (SROC) curve method.

Methods. Data sources were MEDLINE (1966–1999), the Cochrane Library and bibliographies of review and original articles. Studies were included if the absolute numbers of true positive, false negative, true negative and false positive observations were available or could be derived from the data presented; gold standards were described explicitly; and the criteria for a positive ADA result were reported. We constructed the SROC curve based on these extracted data to estimate the test characteristics.

Results. Forty articles were available for analysis. The gold standards used were pleural biopsy histology, microbiological examination of pleural fluid, pleural biopsy and sputum and the patient's clinical course or combinations of these. The sensitivity of ADA reported in the articles ranged from 47% to 100% and the specificity from 50% to 100%. The summary measure of test characteristics derived from the SROC curve was 92% for both sensitivity and specificity.

Conclusions. The test performance of ADA in tuberculous pleural effusion is reasonably good. Measurement of pleural ADA is thus likely to be a useful diagnostic tool for tuberculous pleurisy.

Ann Clin Biochem 2002; 39: 374–381
Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis

Madhukar Pai, Laura L Flores, Nittika Pai, Alan Hubbard, Lee W Riley, and John M Colford Jr

Conventional tests are not always helpful in making a diagnosis of tuberculous meningitis. We did a systematic review and meta-analysis to establish the summary accuracy of nucleic acid amplification (NAA) tests for tuberculous meningitis. We searched six electronic databases and contacted authors, experts, and manufacturers. Measures of diagnostic accuracy were pooled using a random effects model. 49 studies met our inclusion criteria. The summary estimates in 14 studies with commercial NAA tests were: sensitivity 0.66 (95% CI 0.46, 0.66), specificity 0.89 (0.79, 0.94), positive likelihood ratio 35.1 (19.6–64.6), negative likelihood ratio 0.44 (0.33, 0.60), and diagnostic odds ratio 96.4 (42.8, 217.3). In the 35 studies with in-house (“home-brew”) tests, the summary accuracy could not be established with confidence because of wide variability in test accuracy. On current evidence, commercial NAA tests show a potential role in confirming tuberculous meningitis diagnosis, although their overall low sensitivity precludes the use of these tests to rule out tuberculous meningitis with certainty.

Lancet Infect Dis 2001; 3: 63–64

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Adenosine deaminase and interferon gamma measurements for the diagnosis of tuberculous pleurisy: a meta-analysis

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1Divisione Clinizzata di Malattie Respiratorie, Università di Tor Vergata, 2Dipartimento di Epidemiologia, INMI Lazzaro Spallanzani, 1Dipartimento di Medicina Sperimentale and 2Dipartimento Cardiopneumologico delle Università ‘La Sapienza’, Roma, Italy

SUMMARY

OBJECTIVE: To assess Mycobacterium tuberculosis isolation rates in tuberculous effusions are relatively low, several biochemical and immunological markers have been proposed to diagnose tuberculous pleurisy, including adenosine deaminase (ADA) and interferon-gamma (IFN-γ). Here we summarise the literature on ADA and IFN-γ as predictors of tuberculous pleurisy.

METHODS: After a systematic review of English language studies, we used summary receiver operating characteristic curve (SROC) analysis to determine the cumulative diagnostic accuracy of both markers and Bayes’ theorem to calculate post-test probability of disease in settings with different prevalences of tuberculous pleurisy, assessed and reported the quality of primary studies.

RESULTS: From 1978 to November 2000, studies containing sufficient data for the determination of both sensitivity and specificity were 31 on ADA, including 4738 patients, and 13 on IFN-γ, including 1189 patients. SROC curve yielded a maximum joint sensitivity and specificity of 93% for ADA and 96% for IFN-γ. In the setting of tuberculous effusion prevalence of 5%, 25% and 55%, post-test probability of a negative ADA test were 0.4%, 2.4% and 24%, and 0.2%, 1.2% and 17% for a negative IFN-γ test.

CONCLUSION: With the caveat that limitations in the design of the studies summarised here may distort estimates of test performance, ADA and IFN-γ appear to be reasonably accurate at detecting TB pleurisy.

KEY WORDS: meta-analysis; tuberculous pleurisy; adenosine deaminase; interferon type II; laboratory techniques; procedure
Research article

Nucleic acid amplification tests in the diagnosis of tuberculous pleuritis: a systematic review and meta-analysis
Madhukar Pai1, Laura L Flores2, Alan Hubbard3, Lee W Riley2 and John M Colford Jr*1

Abstract

Background: Conventional tests for tuberculous pleuritis have several limitations. A variety of new, rapid tests such as nucleic acid amplification tests – including polymerase chain reaction – have been evaluated in recent times. We conducted a systematic review to determine the accuracy of nucleic acid amplification (NAA) tests in the diagnosis of tuberculous pleuritis.

Methods: A systematic review and meta-analysis of 39 English and Spanish articles (with 40 studies), identified via searches of six electronic databases, hand searching of selected journals, and contact with authors, experts, and test manufacturers. Sensitivity, specificity, and other measures of accuracy were pooled using random effects models. Summary receiver operating characteristic curves were used to summarize overall test performance. Heterogeneity in study results was formally explored using subgroup analyses.

Results: Of the 40 studies included, 26 used in-house ("home-brew") tests, and 14 used commercial tests. Commercial tests had a low overall sensitivity (0.62, 95% confidence interval [CI] 0.43, 0.77), and high specificity (0.98, 95% CI 0.96, 0.98). The positive and negative likelihood ratios for commercial tests were 25.4 (95% CI 16.2, 40.0) and 0.40 (95% CI 0.24, 0.67), respectively. All commercial tests had consistently high specificity estimates; the sensitivity estimates, however, were heterogeneous across studies. With the in-house tests, both sensitivity and specificity estimates were significantly heterogeneous. Clinically meaningful summary estimates could not be determined for in-house tests.

Conclusions: Our results suggest that commercial NAA tests may have a potential role in confirming (ruling in) tuberculous pleuritis. However, these tests have low and variable sensitivity and, therefore, may not be useful in excluding (ruling out) the disease. NAA test results, therefore, cannot replace conventional tests; they need to be interpreted in parallel with clinical findings and results of conventional tests. The accuracy of in-house nucleic acid amplification tests is poorly defined because of heterogeneity in study results. The clinical applicability of in-house NAA tests remains unclear.

Research article

In-house nucleic acid amplification tests for the detection of Mycobacterium tuberculosis in sputum specimens: meta-analysis and meta-regression
Laura L. Flores1,2,3, Madhukar Pai1,2,3, John M Colford Jr4 and Lee W Riley*1

Abstract

Background: More than 200 studies related to nucleic acid amplification (NAA) tests to detect Mycobacterium tuberculosis directly from clinical specimens have appeared in the world literature since this technology was first introduced. NAA tests come as either commercial kits or as tests designed by the reporting investigators themselves (in-house tests). In-house tests vary widely in their accuracy, and factors that contribute to heterogeneity in test accuracy are not well characterized. Here, we used meta-analytical methods, including meta-regression, to identify factors related to study design and assay protocols that affect test accuracy in order to identify those factors associated with high estimates of accuracy.

Results: By searching multiple databases and sources, we identified 2520 potentially relevant citations, and analyzed 84 separate studies from 65 publications that dealt with in-house NAA tests to detect M tuberculosis in sputum samples. Sources of heterogeneity in test accuracy estimates were determined by subgroup and meta-regression analyses. Among 84 studies analyzed, the sensitivity and specificity estimates varied widely; sensitivity varied from 9.4% to 100%, and specificity estimates ranged from 91% to 100%. In the meta-regression analysis, the use of IS6110 as a target, and the use of nested PCR methods appeared to be significantly associated with higher diagnostic accuracy.

Conclusion: Estimates of accuracy of in-house NAA tests for tuberculosis are highly heterogeneous. The use of IS6110 as an amplification target, and the use of nested PCR methods appeared to be associated with higher diagnostic accuracy. However, the substantial heterogeneity in both sensitivity and specificity of the in-house NAA tests rendered clinically useful estimates of test accuracy difficult. Future development of NAA-based tests to detect M tuberculosis from sputum specimens should take into consideration these findings in improving accuracy of in-house NAA tests.
BMC Infectious Diseases

Research article

Bacteriophage-based tests for the detection of Mycobacterium tuberculosis in clinical specimens: a systematic review and meta-analysis

Shrirakpani Kalantri*, Madhukar Pai1,2, Lisa Pascopella3, Lee Riley1 and Arthur Reingold1

Abstract

Background: Sputum microscopy, the most important conventional test for tuberculosis, is specific in settings with high burden of tuberculosis and low prevalence of non-tuberculous mycobacteria. However, the test lacks sensitivity. Although bacteriophage-based tests for tuberculosis have shown promising results, their overall accuracy has not been systematically evaluated.

Methods: We did a systematic review and meta-analysis of published studies to evaluate the accuracy of phage-based tests for the direct detection of M. tuberculosis in clinical specimens. To identify studies, we searched Medline, EMBASE, Web of Science and BIOSIS, and contacted authors, experts and test manufacturers. Thirteen studies, all based on phage amplification method, met our inclusion criteria. Overall accuracy was evaluated using forest plots, summary receiver operating (SROC) curves, and subgroup analyses.

Results: The data suggest that phage-based assays have high specificity (range 0.83 to 1.00), but modest and variable sensitivity (range 0.21 to 0.88). The sensitivity ranged between 0.29 and 0.87 among smear-positive, and 0.13 to 0.78 among smear-negative specimens. The specificity ranged between 0.60 and 0.88 among smear-positive and 0.89 to 0.99 among smear-negative specimens. SROC analyses suggest that overall accuracy of phage-based assays is slightly higher than smear microscopy in direct head-to-head comparisons.

Conclusion: Phage-based assays have high specificity but lower and variable sensitivity. Their performance characteristics are similar to sputum microscopy. Phage assays cannot replace conventional diagnostic tests such as microscopy and culture at this time. Further research is required to identify methods that can enhance the sensitivity of phage-based assays without compromising the high specificity.

Bacteriophage-based assays for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a meta-analysis

Madhukar Pai1,2, Shrirakpani Kalantri*, Lisa Pascopella3, Lee W. Riley4, Arthur L. Reingold4

KEYWORDS
Tuberculosis; Multi-drug resistant tuberculosis; Rifampicin resistance; Bacteriophage; Phage; Diagnosis; Accuracy; Sensitivity and specificity

Abstract: Objective: To summarize, using meta-analysis, the accuracy of bacteriophage-based assays for the detection of rifampicin resistance in Mycobacterium tuberculosis.

Methods: By searching multiple databases and sources we identified a total of 21 studies eligible for meta-analysis. Of these, 14 studies used phage amplification assays (including eight studies on the commercial FASTPlaque™ TB kit), and seven used luciferase reporter phage (LRP) assays. Sensitivity, specificity, and agreement between phage assay and reference standard (e.g. agar proportion method or BACTEC 460) results were the main outcomes of interest.

Results: When performed on culture isolates (n=19 studies), phage assays appear to have relatively high sensitivity and specificity. Eleven of 19 (58%) studies reported sensitivity and specificity estimates ≥95%, and 13 of 19 (68%) studies reported ≥95% agreement with reference standard results. Specificity estimates were slightly lower and more variable than sensitivity; 5 of 19 (26%) studies reported specificity >90%. Only two studies performed phage assays directly on sputum specimens; although one study reported sensitivity and specificity of 100 and 99%, respectively, another reported sensitivity of 80% and specificity of 73%.

Conclusions: Current evidence is largely restricted to the use of phage assays for the detection of rifampicin resistance in culture isolates. When used on culture isolates, these assays appear to have high sensitivity, but variable and slightly lower specificity. In contrast, evidence is lacking on the accuracy of these assays when they are directly applied to sputum specimens. If phage-based assays can be directly used on clinical specimens and if they are shown to have high accuracy, they have the potential to improve the diagnosis of MDR-TB. However, before phage assays can be successfully used in routine practice, several concerns have to be addressed, including unexplained false positives in some studies, potential for contamination and indeterminate results.

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A commercial line probe assay for the rapid detection of rifampicin resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis
Maureen Morgan1, Shripakash Kalantri1,2, Laura Flores3 and Madhuskar Pai1,4

Abstract

Background: Mycobacterium tuberculosis is a leading cause of death worldwide. In multidrug-resistant tuberculosis (MDR-TB) infectiousness is frequently prolonged, jeopardizing efforts to control TB. The conventional tuberculosis drug susceptibility tests are sensitive and specific, but they are not rapid. The INNO-LIPA Rif. TB® (LiPA) is a commercial line probe assay designed to rapidly detect rifampicin resistance, a marker of MDR-TB. Although LiPA has shown promising results, its overall accuracy has not been systematically evaluated.

Methods: We did a systematic review and meta-analysis to evaluate the accuracy of LiPA for the detection of rifampicin-resistant tuberculosis among culture isolates and clinical specimens. We searched Medline, Embase, Web of Science, BIOSIS, and Google Scholar, and contacted authors, experts and the manufacturer. Fifteen studies met our inclusion criteria. Of these, 11 studies used culture isolates, one used clinical specimens, and three used both. We used a summary receiver operating characteristic (SROC) curve and Q² index to perform meta-analysis and summarize diagnostic accuracy.

Results: Twelve of 11 studies that applied LiPA to isolates had sensitivity greater than 95%, and 12 of 14 had specificity of 100%. The four studies that applied LiPA directly to clinical specimens had 100% specificity, and sensitivity that ranged between 80% and 100%. The SROC curve had an area of 0.99 and Q² of 0.97.

Conclusion: LiPA is a highly sensitive and specific test for the detection of rifampicin resistance in culture isolates. The test appears to have relatively lower sensitivity when used directly on clinical specimens. More evidence is needed before LiPA can be used to detect MDR-TB among populations at risk in clinical practice.

Current evidence on diagnostic accuracy of commercially based nucleic acid amplification tests for the diagnosis of pulmonary tuberculosis

S Greco, E Girardi, A Navarra, C Saltini

Background: Even though commercial nucleic acid amplification tests (NAATs) have become the most frequently used molecular tests for laboratory diagnosis of pulmonary tuberculosis (TB), published studies report variable estimates of their diagnostic accuracy. We analyzed the accuracy of commercial NAATs for the diagnosis of pulmonary TB in smear positive and smear negative respiratory samples using culture as a reference standard.

Methods: English language studies reporting data sufficient for calculating sensitivity and specificity of commercial NAATs on smear positive and/or smear negative respiratory samples were included. Meta-regression was used to analyse associations with reference test quality, the prevalence of TB, sample and test type. Predictive values for different levels of pretest probability were quantified using Bayes’ approach.

Results: Sixty-three journal articles published between 1995 and 2004 met the inclusion criteria. Pooled sensitivity and specificity were 0.96 and 0.85 among smear positive samples and 0.66 and 0.98 among smear negative samples. The number of culture media used as reference test, the inclusion of bronchial samples, and the TB prevalence were found to influence the reported accuracy. The test type had no effect on the diagnostic odds ratio but seemed to be correlated with sensitivity or specificity, probably via a threshold effect.

Conclusion: Commercial NAATs can be confidently used to exclude TB in patients with smear positive samples in which environmental mycobacteria infection is suspected and to confirm TB in a proportion of smear negative cases. The methodological characteristics of primary studies have a considerable effect on the reported diagnostic accuracy.
**Fluorescence versus conventional sputum smear microscopy for tuberculosis: a systematic review**

Karen R. Steinert, Megan Henry, Vivienne Ng, Philip C. Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark Perkins, Mohamed Abd Alaziz, Medahir Patel

Most of the world's tuberculosis cases occur in low-income and middle-income countries, where sputum microscopy with a conventional light microscope is the primary method for diagnosing pulmonary tuberculosis. A major shortcoming of conventional microscopy is its relatively low sensitivity compared with culture, especially in patients co-infected with HIV. In high-income countries, fluorescence microscopy rather than conventional microscopy is the standard diagnostic method. Fluorescence microscopy is credited with increased sensitivity and lower work effort, but there is concern that specificity may be lower. We did a systematic review to summarise the accuracy of fluorescence microscopy compared with conventional microscopy. By searching many databases and contacting experts, we identified 45 relevant studies. Sensitivity, specificity, and incremental yield were the outcomes of interest. The results suggest that, overall, fluorescence microscopy is more sensitive than conventional microscopy, and has similar specificity. There is insufficient evidence to determine the value of fluorescence microscopy in HIV-infected individuals. The results of this review provide a point of reference, quantifying the potential benefit of fluorescence microscopy, with which the increased cost and technical complexity of the method can be compared to determine the feasible value of the method under programme conditions.

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**Sputum processing methods to improve the sensitivity of smear microscopy for tuberculosis: a systematic review**

Karen R. Steinert, Vivienne Ng, Megan Henry, Philip C. Hopewell, Andrew Ramsay, Jane Cunningham, Richard Urbanczik, Mark D Perkins, Mohamed Abd Alaziz, Medahir Patel

In low-income and middle-income countries, direct (unconcentrated) sputum smear microscopy is the primary method for diagnosing pulmonary tuberculosis. The method is fast, inexpensive, and specific for Mycobacterium tuberculosis in high incidence areas. The main limitations of direct microscopy are its relatively low sensitivity, especially in individuals co-infected with HIV, and variable quality of the test in programme conditions. Thus, there is a need to identify methods to improve the sensitivity of microscopy. Physical and chemical sputum processing methods, including centrifugation, sedimentation, and bleach, have been studied and found to show promise. We did a systematic review to assess the ability of different processing methods to improve the sensitivity of microscopy. By searching many sources, we identified 83 studies. Overall, by comparison with direct smears, the results suggested that centrifugation with any of several chemical methods (including bleach) is more sensitive, that overnight sedimentation preceded by chemical processing is more sensitive, and that specificity is similar. There were insufficient data to determine the value of sputum processing methods in patients with HIV infection. Operational studies are needed to determine whether the increased sensitivity provided by processing methods is sufficient to offset their increased cost, complexity, and potential biohazards, and to examine their feasibility.
Yield of serial sputum specimen examinations in the diagnosis of pulmonary tuberculosis: a systematic review

S. R. Masri,† A. Ramsay,‡ V. Ng,§ M. Henry,∥ P. C. Hopewell,¶ J. Cunningham,** R. Urbanic†‡‡ M. D. Perkins,*,‡‡ M. A. Aziz,*,‡‡ M. Pali†‡

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SUMMARY

Current international tuberculin (TB) guidelines recommend the microscopic examination of three sputum specimens for acid-fast bacilli in the evaluation of persons suspected of having pulmonary TB. We conducted a systematic review of studies that quantified the diagnostic yield of each of the three sputum specimens. By searching multiple databases and sources, we identified a total of 37 eligible studies. The incremental yield in smear-positive results (in studies using all smear-positive cases as the denominator) and the increase in sensitivity (in studies that used all culture-positive cases as the denominator) of the third specimen were the main outcomes of interest. Although heterogeneity in study methods and results presented challenges for data synthesis, subgroup analyses suggest that the average incremental yield and/or the increase in sensitivity of examining a third specimen ranged between 2% and 5%. Reducing the recommended number of specimens examined from three to two (particularly to two specimens collected on the same day) could benefit TB control programs, and potentially increase case detection for several reasons. A number of operational research issues need to be addressed. Studies examining the most effective and efficient means to utilize current technologies for microscopic examination of sputum would be most useful if they followed an internationally coordinated and standardized approach, both to strengthen the country-specific evidence base and to permit comparison among studies.

KEY WORDS: tuberculous; smear microscopy; incremental yield; acid-fast bacillus; serial sputum specimens

Colorimetric redox-indicator methods for the rapid detection of multidrug resistance in Mycobacterium tuberculosis: a systematic review and meta-analysis

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Received 20 August 2006; revised 23 September 2006; revised 27 October 2006; accepted 29 October 2006

Objectives: With the spread of multidrug-resistant tuberculosis (MDR-TB) there is increasing demand for new accurate and cost-effective tools for rapid drug susceptibility testing (DST), particularly for developing countries. The reference standard method used today for DST is very slow and cumbersome.

Colorimetric assays using redox indicators have been proposed to be used in low-resource countries as rapid alternative culture methods for the detection of resistance especially to rifampicin and isoniazid. These methods appear as promising new tools but their accuracy has not been systematically evaluated.

Methods: We did a meta-analysis to evaluate the accuracy of the colorimetric assays for the detection of rifampicin and isoniazid-resistant tuberculosis among clinical isolates. We searched Medline, PubMed (NCBI), Global health-CAB, EMBASE (Elsevier), ISI Web of Science and IFCC databases and contacted authors if additional information was needed.

Results: Eighteen studies met our inclusion criteria for rifampicin resistance detection and 16 for isoniazid. We used a summary receiver operating characteristic (SROC) curve to perform meta-analysis and summarize diagnostic accuracy. For both drugs, all studies had a sensitivity and specificity that ranged between 88% and 100%.

Conclusions: There is evidence that colorimetric methods are highly sensitive and specific for the rapid detection of MDR-TB. These new tools could offer affordable technologies for TB laboratories especially in places where resources are limited and where the prevalence of MDR-TB is important and make TB control efforts more effective. Additional studies are needed in high MDR prevalence countries and cost-effectiveness analysis to have more evidence on the utility of these methods. Future developments to detect resistance directly from smear-positive sputum specimens should be taken into consideration to speed up the process.
Commercial Serological Antibody Detection Tests for the Diagnosis of Pulmonary Tuberculosis: A Systematic Review

Karen R. Steingart, Megan Henry, Suman Leal, Philip C. Hopewell, Andrew Ramsey, Dick Manzies, Jane Cunningham, Kevin Weldingh, Madhukar Pai

Background

The global tuberculosis epidemic results in nearly 2 million deaths and 9 million new cases of the disease a year. The vast majority of tuberculosis patients live in developing countries, where the diagnosis of tuberculosis relies on the identification of acid-fast bacilli on unprocessed sputum smears using conventional light microscopy. Microscopy has high specificity in tuberculosis-endemic countries, but modest sensitivity which varies among laboratories (range 20% to 80%). Moreover, the sensitivity is poor for paucibacillary disease (e.g., pediatric and HIV-associated tuberculosis). Thus, the development of rapid and accurate new diagnostic tools is imperative. Immune-based tests are potentially suitable for use in low-income countries as some test formats can be performed at the point of care without laboratory equipment. Currently, dozens of distinct commercial antibody detection tests are sold in developing countries. The question is “do they work?”

Methods and Findings

We conducted a systematic review to assess the accuracy of commercial antibody detection tests for the diagnosis of pulmonary tuberculosis. Studies from all countries using culture and/or microscopy were eligible for inclusion. A total of 122 studies (102 patients, 25 control patients) were included. In a comprehensive search, we identified 69 studies. The results demonstrate that (1) overall, commercial tests were more accurate than smear-negative samples; (2) sensitivity is higher in smear-positive than smear-negative samples; (3) in studies of smear-negative patients, AFB-IgG by enzyme-linked immunosorbent assay shows limited sensitivity (range 63% to 85%) and inconsistent specificity (range 73% to 100%); (4) specificity is higher in healthy volunteers than in patients in whom tuberculosis disease is not suspected and subsequently ruled out; and (5) there are insufficient data to determine the accuracy of most commercial tests in smear microscopy-negative patients, as well as their performance in children or persons with HIV infection.

Conclusions

None of the commercial tests evaluated perform well enough to replace sputum smear microscopy in diagnostic practice. These tests have little or no role in the diagnosis of pulmonary tuberculosis. Lack of systematic review in these studies was identified as a concern. It will be important to review the basic science literature evaluating serological tests for the diagnosis of pulmonary tuberculosis to determine whether useful antigens have been described and their potential has not been fully exploited. Activities leading to the discovery of new antigens with immunodiagnostic potential need to be intensified.

A systematic review of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis

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Conventional diagnostic tests for tuberculosis have several limitations and are often unhelpful in establishing the diagnosis of extrapulmonary tuberculosis. Although commercial serological antibody-based tests are available, their usefulness in the diagnosis of extrapulmonary tuberculosis is unknown. A systematic review was conducted to assess the accuracy of commercial serological antibody detection tests for the diagnosis of extrapulmonary tuberculosis. In a comprehensive search, 21 studies that reported data on sensitivity and specificity for extrapulmonary tuberculosis were identified. These studies evaluated seven different commercial tests, with AFB-IgG accounting for 48% of the studies. The studies showed that (1) all commercial tests provided highly variable estimates of sensitivity (range 0.00–1.00) and specificity (range 0.09–1.00) for all extrapulmonary sites combined; (2) the AFB-IgG kit showed highly variable sensitivity (range 0.26–1.00) and specificity (range 0.09–1.00) for all extrapulmonary sites combined; (3) for all tests combined, sensitivity estimates for both lymph node tuberculosis (range 0.23–1.00) and pleural tuberculosis (range 0.26–0.59) were poor and inconsistent; and (4) there were no data to determine the accuracy of the tests in children or in patients with HIV infection, the two groups for which the test would be most useful. At present, commercial antibody detection tests for extrapulmonary tuberculosis have no role in clinical care or research.
Nucleic acid amplification tests for the diagnosis of tuberculous lymphadenitis: a systematic review

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** Summary **

Setting: Lymphadenitis is the most common extra-pulmonary manifestation of tuberculosis (TB). Conventional diagnostic methods such as sputum microscopy and culture are not very accurate for TB lymphadenitis. Nucleic acid amplification tests (NAAT) may offer additional diagnostic benefit.

Objective: To assess, in a systematic review, the performance of NAAT for the diagnosis of tuberculous lymphadenitis.

Design: We performed searches of the literature and identified 36 articles containing 49 comparisons between NAAT and a reference standard for TB lymphadenitis. Sensitivity and specificity estimates from each study were displayed in forest plots and summary receiver operating characteristic (ROC) plots.

Results: Overall study quality was fair, but the quality of reporting was poor in many studies. Estimates of sensitivity and specificity of NAAT were highly heterogeneous across studies, possibly due to variations in populations, study quality and test techniques. Estimates of sensitivity varied between 2% and 100%, and specificity estimates varied between 28% and 100%. Commercial NAAT assays, assays that used more than 20 µl of template and reports containing discrepant analysis provided significantly higher diagnostic accuracy. Blinding, template volume and discrepant analysis may account for some of the observed heterogeneity.

Conclusion: Studies on NAAT for TB lymphadenitis produce highly variable and inconsistent results, precluding the determination of clinically meaningful estimates of accuracy. Study reports are not well standardised and often do not contain enough information. Because both false-positive and false-negative results are possible, NAAT will need to be applied in conjunction with conventional methods and interpreted in the context of clinical suspicion.

Keywords: tuberculosis, lymphadenitis; nucleic acid amplification techniques; NAAT; PCR

A systematic review of rapid diagnostic tests for the detection of tuberculosis infection

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Objectives: To evaluate the effectiveness of available rapid diagnostic tests to identify tuberculosis (TB) infection.

Data sources: Electronic databases were searched from 1975 to August 2003 for tests for active TB and to March 2004 for tests for latent tuberculosis infection (LTBI).

Review methods: Studies were selected and evaluated that (1) tested for LTBI, (2) compared tuberculin skin test (TST) and interferon-γ assays based on ESAT-6 and CFP-10 antigens and (3) provided information on TB exposure or bacille Calmette-Guérin (BCG) vaccination or HIV status. For each test comparison, the sensitivity, specificity and 95% confidence intervals (CIs) were calculated. Sources of heterogeneity were investigated by adding covariates to the standard regression model. The authors examined whether interferon-γ assays were more strongly associated with high versus low TB exposure than TST. Odds ratios (ORs) were calculated for the association between test results and exposure from each study along with their 95% CIs. Within each study, the OR value for one test was divided by that for another to produce a ratio of OR (ROR).

Results: A total of 213 studies were included, providing 369 data sets. A further 19 studies assessing fully automated liquid culture methods were included. Overall, nucleic acid amplification test (NAAT) accuracy was far superior when applied to respiratory samples as opposed to other body fluids. The better quality in-house studies, were, for pulmonary TB, much better at ruling out TB than the commercial tests (higher sensitivity), but were less good at ruling in (lower specificity), but it is not possible to recommend any one over another owing to a lack of direct test comparisons. The specificity of NAAT tests was high when applied to body fluids, for example for TB meningitis and pleural TB, but sensitivity was poor, indicating that these tests cannot be used reliably to rule out TB. High specificity estimates suggest that NAAT tests should be the first-line test for ruling in TB meningitis, but that they need to be combined with the results of other tests in order to rule out disease. Evidence for NAAT tests in other forms of TB and for phase-based tests is significantly less prolific than for those above and further research is needed to establish accuracy. There is no evidence to support the use of adenosine deaminase (ADA) tests for diagnosis of pulmonary TB; however, there is considerable evidence to support their use for diagnosis of pleural TB and to a slightly lesser extent for TB meningitis. Anti-TB antibody test performance was universally poor, regardless of type of TB. Fully automated liquid culture methods were superior to culture on solid media, in terms of their speed and their precision. In total, 13 studies were included. Assays based on RD1-specific antigens, ESAT-6 and CFP-10, correlated better with intensity of exposure, and therefore are more likely than TST/purified protein derivative (PPD)-based assays to detect LTBI accurately. An additional advantage is that they are more likely to be independent of BCG vaccination status and HIV status.

Conclusions: The NAAT tests provide a reliable way of increasing the specificity of diagnosis (ruling in

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Background: Until recently, the tuberculin skin test was the only test for detecting latent tuberculosis (TB) infection, but 2 ex vivo interferon-γ release assays (IGRA) are now commercially licensed.

Purpose: To estimate sensitivity, specificity, and reproducibility of IGRA (commercial) or research versions of Quantiferon (QFT) and Elispot for diagnosing latent TB infection in healthy and immune-suppressed persons.

Data Sources: The authors searched MEDLINE and reviewed citations of all original articles and reviews for studies published in English.

Study Selection: Studies evaluated IGRA using Mycobacterium tuberculosis-specific antigens (PDT antigens) and overnight (16- to 24-h) incubation times. Reference standards had to be clearly defined without knowledge of test results.

Data Extraction and Quality Assessment: Specific criteria for quality assessment were developed for sensitivity, specificity, and reproducibility.

Data Synthesis: When newly diagnosed active TB was used as a surrogate for latent TB infection, sensitivity of all tests was suboptimal, although it was higher with Elispot. No test distinguishes active TB from latent TB. Sensitivity of the tuberculin skin test and IGRA was similar in persons who were categorized into clinical gradients of exposure. Pooled specificity was 97.7% (95% CI, 96% to 99%) and 92.5% (CI, 86% to 99%) for QFT and for Elispot, respectively. Both assays were more specific than the tuberculin skin test in samples vaccinated with bacille Calmette-Guérin. Elispot was more sensitive than the tuberculin skin test in 3 studies of immune-compromised samples. Discordant tuberculin skin test and IGRA reactions were frequent and largely unexplained, although some may be related to varied definitions of positive test results. Resolution of IGRA results from positive to negative was common in 2 studies in which it was assessed.

Limitations: Most studies used cross-sectional designs with the inherent limitation of no gold standard for latent TB infection, and most involved small samples with a widely varying likelihood of true-positive and false-positive test results. There is insufficient evidence on IGRA performance in children, immune-compromised persons, and the elderly.

Conclusions: New IGRA show considerable promise and have excellent specificity. Additional studies are needed to better define their performance in high-risk populations and in serial testing. Longitudinal studies are needed to predict the predictive value of IGRA.


Diagnostic Value of Interferon-γ in Tuberculous Pleurisy*

A Metaanalysis

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Background: Conventional tests are not always helpful in making a diagnosis of tuberculous pleurisy. Many studies have investigated the usefulness of interferon (IFN)-γ measurements in pleural fluid for the early diagnosis of tuberculous pleurisy. We conducted a metaanalysis to determine the accuracy of IFN-γ measurements in the diagnosis of tuberculous pleurisy.

Methods: After a systematic review of English-language studies, sensitivity, specificity, and other measures of accuracy of IFN-γ concentrations in the diagnosis of pleural effusion were pooled using random-effects models. Summary receiver operating characteristic curves were used to summarize overall test performance.

Results: Twenty-two studies met our inclusion criteria. The summary estimates for IFN-γ in the diagnosis of tuberculous pleurisy in the studies included were as follows: sensitivity, 0.89 (95% confidence interval [CI], 0.87 to 0.91); specificity, 0.87 (95% CI, 0.80 to 0.90); positive likelihood ratio, 23.45 (95% CI, 17.31 to 31.78); negative likelihood ratio, 0.11 (95% CI, 0.07 to 0.16); and diagnostic odds ratio, 272.7 (95% CI, 147.3 to 504.2).

Conclusions: IFN-γ determination is a sensitive and specific test for the diagnosis of tuberculous pleurisy. The measurement of IFN-γ levels in pleural effusion is thus likely to be a useful tool for diagnosing tuberculous pleurisy. The results of IFN-γ assays should be interpreted in parallel with clinical findings and the results of conventional tests. (CHEST 2007; 131:1133-1141)