Diagnostic accuracy of nucleic acid amplification tests for tuberculous meningitis: a systematic review and meta-analysis

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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 0·56 (95% CI 0·46, 0·66), specificity 0·98 (0·97, 0·99), positive likelihood ratio 35·1 (19·0, 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.

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Tuberculosis is a public-health problem of global importance. According to the WHO, more than 8 million people develop tuberculosis each year.[1] With the rise in prevalence of HIV infection, extrapulmonary and disseminated forms of tuberculosis are increasingly seen. In England and Wales, 38% of tuberculosis cases are extrapulmonary.[2] In the USA, nearly 20% of tuberculosis cases are extrapulmonary.[3] Tuberculous meningitis is a potentially fatal form of extrapulmonary tuberculosis with serious long-term consequences.[4–7]

In developed countries such as the USA, tuberculosis has become primarily an inner-city disease.[4] Tuberculous meningitis among inner-city residents is a devastating disease with high fatality. Porkert et al[4] reported a fatality rate of 41% among treated tuberculous meningitis patients in a public hospital in Atlanta. Studies from developing countries have reported fatality rates as high as 44–69%.[5–7] Because of the non-specific clinical features, and the deadly consequences of a missed diagnosis, accurate, early confirmation of diagnosis is an essential component in the management of tuberculous meningitis. Furthermore, there are potentially serious side-effects as well as costs associated with the treatment of patients incorrectly diagnosed.

The diagnostic workup for tuberculous meningitis involves detection of acid-fast bacilli (AFB) in the cerebrospinal fluid (CSF) by microscopy (smear) and culture. These conventional tests are often unhelpful in making the diagnosis since tuberculous meningitis is a paucibacillary form of tuberculosis. Microscopy, although rapid and inexpensive, has very low sensitivity (10–20%).[8,9] Culture, another established method, is not very sensitive (<50%), and results are not available for weeks.[8,9] Further, a diagnosis of tuberculous meningitis cannot be made nor excluded on the basis of clinical features.[8,9] There is some evidence that a combination of clinical data (age, duration of history) and simple laboratory data (white blood cell count, total CSF white cell count, and CSF neutrophil proportion) might help in the diagnosis of adult tuberculous meningitis.[8] In the context of these limitations, nucleic acid amplification (NAA) tests have emerged with the intended goal of enabling clinicians to make a rapid and accurate diagnosis.[10] PCR is the best-known NAA test. All NAA tests amplify target nucleic acid regions that uniquely identify the *Mycobacterium tuberculosis* complex. An important advantage of NAA tests is the rapidity by which the results can be obtained—about 3–6 h from receipt of specimen.[10]

NAA tests are categorised as commercial or in-house ("home-brew"). A recent report from the USA indicated that NAA tests were used in hospitals, health departments, and independent laboratories.[11] Most (>90%) laboratories used commercial kits such as the Amplicor *M tuberculosis* tests (Roche Molecular Systems, Branchburg, NJ, USA), and the Amplified *M tuberculosis* Direct Test (MTD; Gen-Probe Inc, San Diego, CA, USA).[11] In the USA, the Amplicor and the MTD tests are licensed by the Food and Drug Administration (FDA) for use in smear-positive respiratory specimens.[12] In 1999, the FDA approved an enhanced Gen-Probe MTD test for use in smear-negative respiratory specimens.[12] Another test, the LCx kit (Abbott Laboratories, Abbott Park, IL) has been recently discontinued. No commercial test is licensed for use in non-respiratory specimens. The costs of commercial NAA tests vary (list price US$25–50 per test). The cost of in-house PCR has been estimated to be about $15 per test.[13]

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The accuracy of NAA tests for tuberculosis has been extensively studied since the early 1990s. However, the exact role of these tests remains controversial.[10,12,14] According to the US Centers for Disease Control and Prevention (CDC)[12] and the American Thoracic Society (ATS),[14] NAA tests improve diagnostic certainty but do not replace microscopy and culture. NAA tests can greatly increase confidence in the clinical diagnosis pending culture results. Patients with the greatest potential to benefit from the NAA tests are those who are smear-negative. In these patients, if the NAA test is positive, further specimens should be tested with NAA tests; a patient can be presumed to have tuberculosis if a subsequent specimen is NAA test-positive.[12] Thus, in these patients NAA tests can lead to earlier diagnosis and rapid initiation of therapy. The ATS/CDC guidelines do not recommend the use of NAA tests for monitoring treatment response since the tests can amplify DNA from dead bacilli.[14]

Although the CDC has provided an algorithm for NAA testing of respiratory specimens,[12] they offer no recommendation for specimens such as CSF. We did a systematic review to (1) establish the overall accuracy of NAA tests for tuberculous meningitis; (2) identify factors associated with heterogeneity of findings between studies; and (3) assess the effect of study and test characteristics on diagnostic accuracy.

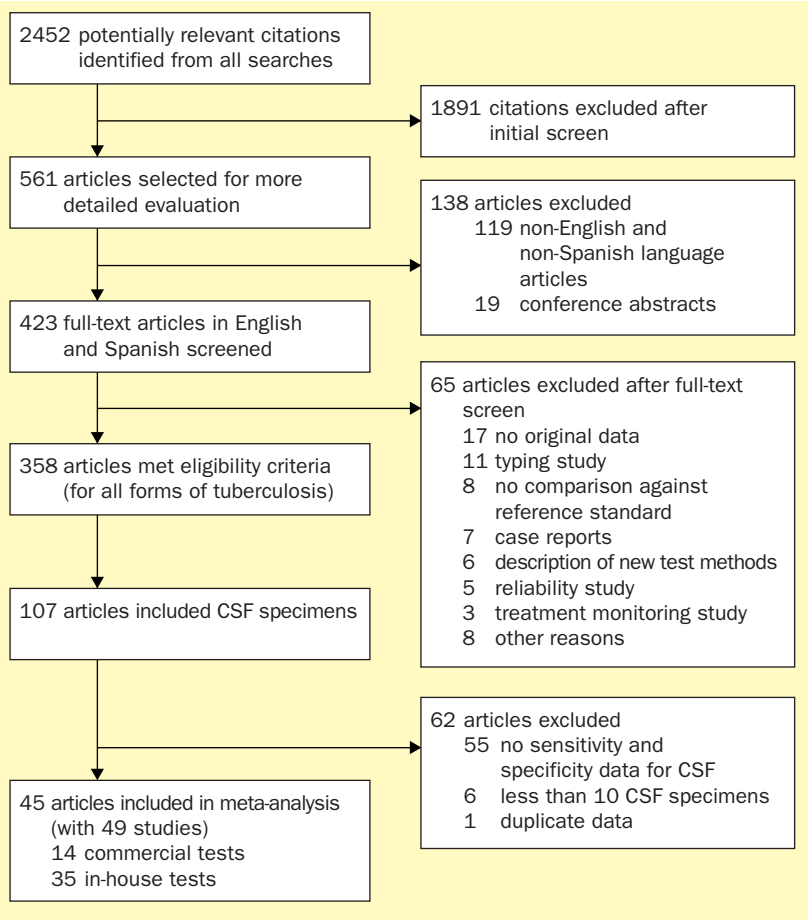

Figure 1. Study selection process and reasons for exclusion of studies.

Methods

Search strategy and selection criteria

We searched the following electronic databases: Medline (1985–2002), Embase (1988–2002), Web of Science (1990–2002), BIOSIS (1993–2002), Cochrane Library (2002; issue 2), and LILACS (1990–2002). All searches were up to date as of August 2002. The search terms were "tuberculosis", "*Mycobacterium tuberculosis*", "nucleic acid amplification techniques", "direct amplification test", "polymerase chain reaction", "ligase chain reaction", "molecular diagnostic techniques", "sensitivity and specificity", "accuracy", or "predictive value". We contacted experts in the specialty, and searched the reference lists from primary and review articles. We obtained lists of studies from companies that manufacture commercial tests. We sought unpublished data from these companies, but none were provided in response to our requests. Although we did not impose language restrictions while searching, only English and Spanish articles were reviewed. Conference abstracts were excluded because they universally contained inadequate data to permit evaluation.

Our search strategy aimed to include all available studies on NAA tests for direct detection of *M tuberculosis* in CSF specimens. For inclusion, the studies had to report a comparison of an NAA test against a reference standard, provide data necessary for the computation of both sensitivity and specificity, and include at least ten CSF specimens in the study (since very small studies may be vulnerable to selection bias).[15]

Two reviewers (MP and LLF) independently judged study eligibility. Disagreements were resolved by consensus. A list of excluded studies, along with the reasons for exclusion, is available from the authors on request.

Data abstraction and quality assessment

The final set of English articles was assessed independently by two reviewers (MP and LLF), who extracted the data using a piloted data extraction form. One reviewer (LLF) assessed all Spanish articles. The reviewers were blinded to publication details. Disagreements were resolved by consensus. Data retrieved from the reports included methodological quality, participant characteristics, test methods, and outcome data.

Since tuberculous meningitis has no single definitive reference standard, we included studies with all types of reference standards and grouped them into three classes. Class one was microbiological diagnosis alone (culture alone; culture and/or microscopy), class two was a combination of microbiological and clinical diagnosis, and class three was clinical diagnosis, response to therapy,

Table 1. Study characteristics and methodological quality of included studies, before and after contacting authors of primary studies

Study characteristic	After obtaining additional information from authors of primary studies			Before contacting authors
	Commercial tests (n=14) %	In-house tests (n=35) %	All studies (n=49) %	All studies (n=49) %
Patient population				
General or primary care population	29	6	12	0
Referred population	64	86	80	88
Unknown	7	8	8	12
Study design				
Cross-sectional	100	46	61	63
Case-control	0	54	39	37
Verification of NAA test results with reference standard				
Complete	100	91	94	94
Partial	0	9	6	6
Blinded assessment of NAA test and reference standard results				
Double blind	36	34	35	12
Single blind	21	26	24	14
Unblinded	7	11	10	0
Unknown	36	29	31	74
Sampling of patients				
Consecutive or random	43	52	49	18
Non-consecutive and non-random	36	14	20	6
Unknown	21	34	31	76
Data collection strategy				
Prospective	86	51	61	51
Retrospective	0	6	4	0
Both	7	11	10	2
Unknown	7	32	25	47
Year of publication				
Before 1996	0	51	37	37
1996 or after	100	49	63	63
Study size				
<10 TBM positive CSF specimens	86	49	59	59
10+ TBM positive CSF specimens	14	51	41	41
Study funded by industry?				
Yes	57	0	16	8
No	43	100	84	92
Reference standard*				
Class 1	65	37	45	45
Class 2	21	37	33	33
Class 3	14	26	22	22

*Reference standards: class 1=microbiological diagnosis; class 2=microbiological plus clinical diagnosis; class 3=clinical diagnosis, response to therapy, and other laboratory tests. TBM=tuberculous meningitis.

and other laboratory tests. Class three included data such as clinical features, CSF analyses, imaging studies, history of contact with tuberculosis, presence of extrameningeal tuberculosis, and response to tuberculosis therapy. Some of the studies compared NAA tests against more than one reference standard. We used a hierarchical approach to select one pair of sensitivity and specificity estimates from each study: if a study used a class two reference standard, those data were preferentially included. If a class two reference standard was not used, data from a comparison against a class one reference standard were included. If neither of the above two reference standards were used, data from a comparison against a class three reference standard were included. Since discrepant analysis (where discordant results between test and culture results are resolved, post-hoc, using clinical data) may be a potential source of bias in NAA test assessments, we preferentially included unresolved data where available.

Two blinded reviewers (MP and LLF) assessed the quality of the studies using methods described by Lijmer et al.[16] The following five quality criteria were evaluated: study design (case-control versus cross-sectional), blinding (single/double blinded versus unblinded interpretation of test and reference standard results), potential for verification bias (complete versus partial verification of test results by reference standard), patient sampling method (consecutive/random versus non-consecutive and non-random), and prospective data collection (ie, prospective patient recruitment). Verification bias may occur if only NAA test-positive specimens are subject to the reference standard (partial verification).

Statistical analysis

We used standard methods recommended for meta-analyses of diagnostic studies.[15,17–19] Analyses were done using Meta-

Table 2. Description of studies in the meta-analysis and measures of test accuracy*

Study, year	Study design	Double or single blind	Consecutive or random sample	Prospective data collection	Type of test† (target sequence)	Reference standard‡	Number of specimens with TBM/number without TBM	Sensitivity (95% CI)	Specificity (95% CI)
Commercial tests (n=14)									
Pfyffer et al 1996[28]	CS	Unknown	Unknown	Yes	MTD (rRNA)	2	6/48	1·00 (0·54, 1·00)	0·96 (0·85, 0·99)
Ehlers et al 1996[29]	CS	Yes	No	No	MTD (rRNA)	3	6/45	0·67 (0·24, 0·94)	0·98 (0·87, 1·00)
D'Amato et al 1996[30]	CS	Unknown	Unknown	Unknown	Amplicor (16s rRNA)	1	2/79	0·50 (0·03, 0·97)	0·99 (0·92, 1·00)
Gamboa et al 1997[31]	CS	Yes	No	Yes	MTD (rRNA)	1	8/14	0·63 (0·26, 0·90)	1·00 (0·77, 1·00)
Gamboa et al 1997[32]	CS	Yes	No	Yes	MTD (rRNA)	1	8/9	0·63 (0·26, 0·90)	1·00 (0·66, 1·00)
Shah et al 1998[33]	CS	Unknown	Yes	Yes	Amplicor (16s rRNA)	1	3/389	0·67 (0·13, 0·98)	1·00 (0·98, 1·00)
Lang et al 1998[34]	CS	Unknown	No	Yes	MTD (rRNA)	2	24/60	0·33 (0·17, 0·55)	1·00 (0·94, 1·00)
Reischl et al 1998[35]	CS	Yes	Yes	Yes	Cobas Amplicor (16s rRNA)	1	3/74	0·67 (0·13, 0·98)	0·99 (0·92, 1·00)
Bonington et al 1998[36]	CS	Yes	Yes	Yes	Amplicor (16s rRNA)	1	8/29	0·88 (0·47, 0·99)	1·00 (0·88, 1·00)
Bonington et al 2000[37]	CS	Yes	Yes	Yes	Cobas Amplicor (16s rRNA)	1	8/29	0·50 (0·18, 0·82)	1·00 (0·88, 1·00)
Brienze et al 2001[38]	CS	Yes	Yes	Yes	Amplicor (16s rRNA)	2	11/17	0·36 (0·13, 0·68)	0·94 (0·69, 1·00)
Morcillo et al 2001[39]	CS	Yes	Yes	Yes	LCx (38 kDa)	1	9/77	0·67 (0·31, 0·91)	1·00 (0·95, 1·00)
Baker et al 2002[40]	CS	No	No	Yes	MTD (rRNA)	1	5/24	1·00 (0·48, 1·00)	1·00 (0·86, 1·00)
Rajo et al 2002[41]	CS	Unknown	Unknown	Yes	LCx (38 kDa)	3	9/78	0·56 (0·23, 0·84)	1·00 (0·95, 1·00)
In-house PCR tests (n=35)									
Kaneko et al 1990[42]	CC	Unknown	Unknown	Unknown	PCR (MPB64)	3	6/20	0·83 (0·37, 0·99)	1·00 (0·83, 1·00)
Shankar et al 1991[43]	CC	Yes	Yes	Yes	PCR (MPB64)	3	34/51	0·65 (0·47, 0·80)	0·88 (0·76, 0·95)
Kolk et al 1992[44]	CS	Yes	No	Unknown	PCR (IS986)	1	7/95	0·86 (0·42, 0·99)	0·72 (0·61, 0·80)
Donald et al 1993[45]	CC	Unknown	Unknown	Yes	PCR (not reported)	3	43/24	0·63 (0·47, 0·77)	1·00 (0·86, 1·00)
Miyazaki et al 1993[46]	CS	Unknown	Unknown	Unknown	Nested PCR (38 kDa)	1	3/29	1·00 (0·29, 1·00)	0·86 (0·68, 0·95)
Machado et al 1994[47]	CC	Unknown	Unknown	Unknown	PCR (65 kDa)	3	10/10	0·70 (0·36, 0·92)	1·00 (0·69, 1·00)
Kox et al 1994[48]	CS	Unknown	Unknown	Unknown	PCR (IS6110)	1	2/13	1·00 (0·16, 1·00)	0·92 (0·62, 1·00)
Verma et al 1994[49]	CS	Yes	Unknown	Unknown	PCR (23s rRNA)	3	10/11	1·00 (0·69, 1·00)	0·82 (0·48, 0·97)
Folgueira et al 1994[50]	CC	Yes	Yes	Yes	PCR (IS6110)	2	9/14	1·00 (0·66, 1·00)	1·00 (0·77, 1·00)
Lee et al 1994[51]	CC	No	Yes	Yes	PCR (IS6110)	2	6/21	1·00 (0·54, 1·00)	0·38 (0·19, 0·61)
Lee et al 1994[51]	CC	No	Yes	Yes	PCR (65 kDa)	2	6/21	1·00 (0·54, 1·00)	0·62 (0·39, 0·81)
Lee et al 1994[51]	CC	No	Yes	Yes	PCR (MPB64)	2	6/21	1·00 (0·54, 1·00)	0·90 (0·68, 0·98)
Liu et al 1994[52]	CC	Unknown	Unknown	Yes	Nested PCR (MPB64)	1	6/79	1·00 (0·54, 1·00)	1·00 (0·95, 1·00)
Amin et al 1994[53]	CC	Yes	Unknown	Unknown	PCR (5·6 Kb Alu)	3	25/25	0·88 (0·68, 0·97)	0·96 (0·78, 1·00)

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Table 2. Description of studies in the meta-analysis and measures of test accuracy* (continued)

Study, year	Study design	Double or single blind	Consecutive or random sample	Prospective data collection	Type of test† (target sequence)	Reference standard‡	Number of specimens with TBM/number without TBM	Sensitivity (95% CI)	Specificity (95% CI)
Lin et al 1995[54]	CC	Unknown	Unknown	Yes	PCR (MPB64)	3	10/27	0·80 (0·44, 0·96)	0·96 (0·79, 1·00)
Miorner et al 1995[55]	CC	Yes	No	No	PCR (IS6110)	2	33/34	0·55 (0·37, 0·71)	0·94 (0·79, 0·99)
Kox et al 1995[56]	CS	Yes	Unknown	Unknown	PCR (IS6110)	2	23/19	0·48 (0·28, 0·69)	1·00 (0·82, 1·00)
Scarpellini et al 1995[57]	CC	Yes	Yes	No	Nested PCR (IS6110)	2	12/24	1·00 (0·74, 1·00)	1·00 (0·86, 1·00)
Seth et al 1996[58]	CC	Yes	Yes	Yes	PCR (MPB64)	3	24/49	0·88 (0·67, 0·97)	0·94 (0·82, 0·98)
Chan et al 1996[59]	CS	Unknown	Yes	Unknown	Nested PCR (IS6110)	1	17/356	0·53 (0·29, 0·76)	0·99 (0·97, 1·00)
Kirschner et al 1996[60]	CS	Unknown	No	Yes	PCR (16s rRNA)	1	3/33	1·00 (0·29, 1·00)	0·97 (0·83, 1·00)
Nguyen et al 1996[61]	CS	Yes	Yes	Yes	PCR (IS6110)	2	99/37	0·32 (0·23, 0·43)	1·00 (0·91, 1·00)
Smith et al 1996[62]	CC	Yes	No	Yes	PCR (IS6110)	2	4/13	0·25 (0·01, 0·78)	1·00 (0·75, 1·00)
Rossetti et al 1997[63]	CS	Yes	Unknown	Unknown	PCR (IS6110)	1	4/50	0·75 (0·22, 0·99)	0·78 (0·64, 0·88)
Jatana et al 2000[64]	CC	Unknown	Unknown	Unknown	PCR (IS6110)	3	27/26	1·00 (0·87, 1·00)	1·00 (0·87, 1·00)
Martins et al 2000[65]	CS	Yes	Yes	No	Nested PCR (MPB64)	1	1/16	1·00 (0·03, 1·00)	0·88 (0·61, 0·98)
Caws et al 2000[66]	CS	No	Yes	Yes	PCR (IS6110)	1	4/105	0·75 (0·22, 0·99)	0·94 (0·88, 0·98)
Portillo-Gomez et al 2000[67]	CC	Yes	Yes	Yes	PCR (IS6110)	2	33/113	0·94 (0·78, 0·99)	1·00 (0·97, 1·00)
Moguel et al 2000[68]	CS	Yes	Yes	Yes	PCR (285 bp)	1	1/14	1·00 (0·03, 1·00)	0·86 (0·56, 0·97)
Gunisha et al 2001[69]	CS	Yes	No	Yes	PCR (IS6110)	1	2/38	0·00 (0·00, 0·84)	0·97 (0·85, 1·00)
Narayanan et al 2001[70]	CS	Yes	Yes	No	PCR (IS6110)	1	20/8	0·95 (0·73, 1·00)	0·75 (0·36, 0·95)
Narayanan et al 2001[70]	CS	Yes	Yes	No	PCR (TRC4)	1	20/8	1·00 (0·83, 1·00)	0·75 (0·36, 0·95)
Correa et al 2001[71]	CC	Yes	Yes	Yes	PCR (IS6110)	2	9/37	0·89 (0·51, 0·99)	0·95 (0·81, 0·99)
Brienze et al 2001[38]	CS	Yes	Yes	Yes	Nested PCR (MPB64)	2	15/50	0·53 (0·28, ·78)	1·00 (0·93, 1·00)
Sumi et al 2002[72]	CC	Yes	Yes	No	PCR (IS6110)	2	45/45	0·47 (0·32, 0·62)	1·00 (0·92, 1·00)

*Incorporates additional information provided by authors of the primary studies. †Type of test: Amplicor & Cobas Amplicor (Roche Molecular Systems, Branchburg, NJ, USA); MTD (Gen-Probe Inc, San Diego, CA, USA); LCx (Abbott Laboratories, Abbott Park, IL, USA). ‡Reference standards: class 1=microbiological diagnosis, class 2=microbiological plus clinical diagnosis, class 3=clinical diagnosis, response to therapy, and other laboratory tests. TBM=tuberculous meningitis. CI=confidence interval. CC=case-control. CS=cross-sectional.

Test,[20] and STATA version 7 (Stata Corporation, TX, USA) using methods described by Sterne et al.[21] For each study we computed measures of test accuracy using standard methods: sensitivity, specificity, positive likelihood ratio (LR+), negative likelihood ratio (LR–), and diagnostic odds ratio (DOR).[17–19,22] These measures were pooled using the random effects model.[17,18]

Each study in the meta-analysis contributed a pair of numbers: sensitivity and specificity. Since these measures are correlated, we summarised their joint distribution using a summary receiver operating characteristic (SROC) curve.[23] Unlike a traditional ROC plot that explores the effect of varying thresholds (cut points for determining test positives) on sensitivity and specificity in a single study, each data point in the SROC plot represents a separate study. The SROC curve presents a global summary of test performance, and shows the trade off between sensitivity and specificity. A symmetric curve suggests that the variability in accuracy between studies is explained, in part, by differences in thresholds used by the studies. It also suggests a common,

homogeneous underlying DOR that does not change with the diagnostic threshold.[17,18,23] The area under the SROC curve is a global measure of overall performance. An area under the curve of 1 indicates perfect discriminatory ability.[17,18,23]

Since the SROC curve and the DOR are not easy to interpret and use in clinical practice,[18] and since likelihood ratios are considered more clinically meaningful,[18,22] we present summary likelihood ratios as our primary measure of diagnostic accuracy. Likelihood ratios of greater than 10 or less than 0·1 generate large and often conclusive shifts from pretest to post-test probability (indicating high accuracy).[22]

Heterogeneity in meta-analysis refers to the degree of variability in study results. An exploration of the reasons for heterogeneity rather than computation of a single summary measure is an important goal of meta-analysis.[24] We investigated heterogeneity using stratified (subgroup) analyses.[25] The following factors were specified a priori as potential sources of heterogeneity: commercial versus in-house tests, and components of study quality; study design, verification, blinded interpretation of NAA test and reference standard, patient sampling, and data collection strategy.

Since test accuracy is affected by the reference standard used,[15] we examined the effect of reference standard on test accuracy with sensitivity analyses—by computing the summary measures with and without studies that used class three (clinical) reference standards. Finally, since publication bias is an important concern with meta-analyses of diagnostic studies,[26] we used funnel plots and the Egger test[27] to assess potential publication bias.

Table 3. Summary measures of test accuracy for all studies, commercial, and in-house tests

Test property	Summary measure of test accuracy* (95% CI)	Test for heterogeneity† p value
All studies (n=49)		
Sensitivity	0·71 (0·63, 0·77)	<0·001
Specificity	0·95 (0·92, 0·97)	<0·001
Positive likelihood ratio (LR+)	15·4 (9·6, 24·9)	<0·001
Negative likelihood ratio (LR–)	0·25 (0·15, 0·39)	<0·001
Diagnostic odds ratio (DOR)	59·4 (40·6, 86·9)	0·43
Commercial tests (n=14)		
Sensitivity	0·56 (0·46, 0·66)	0·10
Specificity	0·98 (0·97, 0·99)	0·10
Positive likelihood ratio (LR+)	35·1 (19·0, 64·6)	0·78
Negative likelihood ratio (LR–)	0·44 (0·33, 0·60)	0·07
Diagnostic odds ratio (DOR)	96·4 (42·8, 217·3)	0·75
In-house tests (n=35)		
Sensitivity	0·76 (0·67, 0·83)	<0·001
Specificity	0·92 (0·88, 0·95)	<0·001
Positive likelihood ratio (LR+)	11·5 (6·8, 19·7)	<0·001
Negative likelihood ratio (LR–)	0·21 (0·11, 0·40)	<0·001
Diagnostic odds ratio (DOR)	54·8 (34·4, 87·2)	0·28

*Random effects model. †χ^2 test for heterogeneity. CI=confidence interval

Results

Figure 1 outlines our study selection process. 45 articles[28–72] were included in the analysis. Three articles were in Spanish.[39,68,71] Three articles reported assessments of more than one NAA test against a common reference standard.[38,51,70] Each such test comparison was counted as a separate study. Thus, the total number of test comparisons (hereafter referred to as studies) was 49. Of these, 14 (29%) were studies of commercial tests,[28–41] and 35 (71%) were of in-house tests.[38,42–72] The average (median) size of each study in the meta-analysis was 42 (specimens or subjects), with a range of 15 to 392.

Quality of reporting and study characteristics

The average inter-rater agreement between the two reviewers for items in the quality checklist was 0·83. Our initial data were affected by the poor quality of reporting in the primary studies. To overcome this problem, we contacted 41 of 45 authors, and additional data were obtained from 24 authors. Table 1 summarises the methodological characteristics of the 49 studies, before and after obtaining additional information from the authors. It shows the variability in study quality and the methodological differences between studies that assessed commercial and in-house tests. Studies with commercial tests were all cross-sectional and 86% collected data prospectively. By comparison, only about half the studies with in-house tests were cross-sectional and prospective. All the studies with commercial tests had been published after 1996 and 86% involved small numbers (<10) of specimens from confirmed tuberculous meningitis patients. About half of the studies with in-house tests had been published before 1996, and about 49% involved small numbers of specimens from tuberculous meningitis patients.

Studies that assessed commercial and in-house tests also differed in laboratory characteristics. None of the commercial tests used the IS6110 target sequence. Also, none of the commercial tests used nested PCR or phenol-chloroform for DNA extraction. Of the 14 studies with commercial tests, six evaluated the Amplicor test,[30,33,35–38] six evaluated the MTD test,[28,29,31,32,34,40] and two studies used the LCx test.[39,41] The relevant study and test characteristics for each of the studies are shown, along with sensitivity and specificity estimates, in table 2. This table shows the variability in study quality, and variability in test methods and reference standards used. Among the in-house tests, a range of PCR protocols was used: 15 different methods (or combination of methods) were used for DNA extraction, and eight different target sequences were amplified.

Overall diagnostic accuracy

Our initial meta-analysis included all 49 studies (table 3). All summary measures except DOR were significantly heterogeneous and not meaningfully summarised. We therefore explored reasons for heterogeneity using subgroup analyses. Since commercial tests, by definition, are standardised by comparison with in-house tests, we did a separate meta-analysis for each subgroup.

Diagnostic accuracy of commercial tests

Figure 2A shows the forest plot of sensitivity and specificity estimates from 14 studies that used commercial tests. Almost all studies show nearly perfect specificity estimates. By contrast, sensitivity estimates were lower and more variable (range 0·33–1·0). Figure 3A shows the SROC curve for the commercial tests. The curve does not show a clear ROC-type of trade off between sensitivity and specificity; specificity estimates are very high whereas sensitivity estimates are variable. Table 3 shows the results of the meta-analysis. The summary estimate of specificity was high (0·98) while sensitivity was not (0·56). The summary measures for commercial tests were considerably less heterogeneous than the summary measures from the meta-analysis of all 49 studies. Sensitivity analysis showed that the summary measures computed after excluding two studies[29,41] with class three (clinical) reference standards (sensitivity 0·57, specificity 0·98) were almost identical to those computed with all 14 studies (sensitivity 0·56, specificity 0·98).

Diagnostic accuracy of in-house tests

Figure 2B shows the forest plot of sensitivity and specificity estimates from 35 studies that used in-house tests. Again, sensitivity estimates were lower and variable (range 0–1·0) compared with specificity (range 0·38–1·0). All summary measures except DOR were grossly heterogeneous (table 3). The SROC curve (figure 3B) was symmetric, showing a clear trade off between sensitivity and specificity. Overall, the significant heterogeneity in sensitivity and specificity estimates precluded the determination of clinically useful summary measures.

Exploration of heterogeneity and publication bias

We did stratified analyses in the in-house tests subgroup to identify factors associated with heterogeneity (table 4). Among the quality factors assessed, study design and blinding seemed to be associated with DOR. Case-control studies produced DOR estimates that were twofold higher than cross-sectional studies. Studies that did not use a blinded interpretation of the results of NAA test or reference test produced DOR estimates that were about 1·8 times greater than studies that were single or

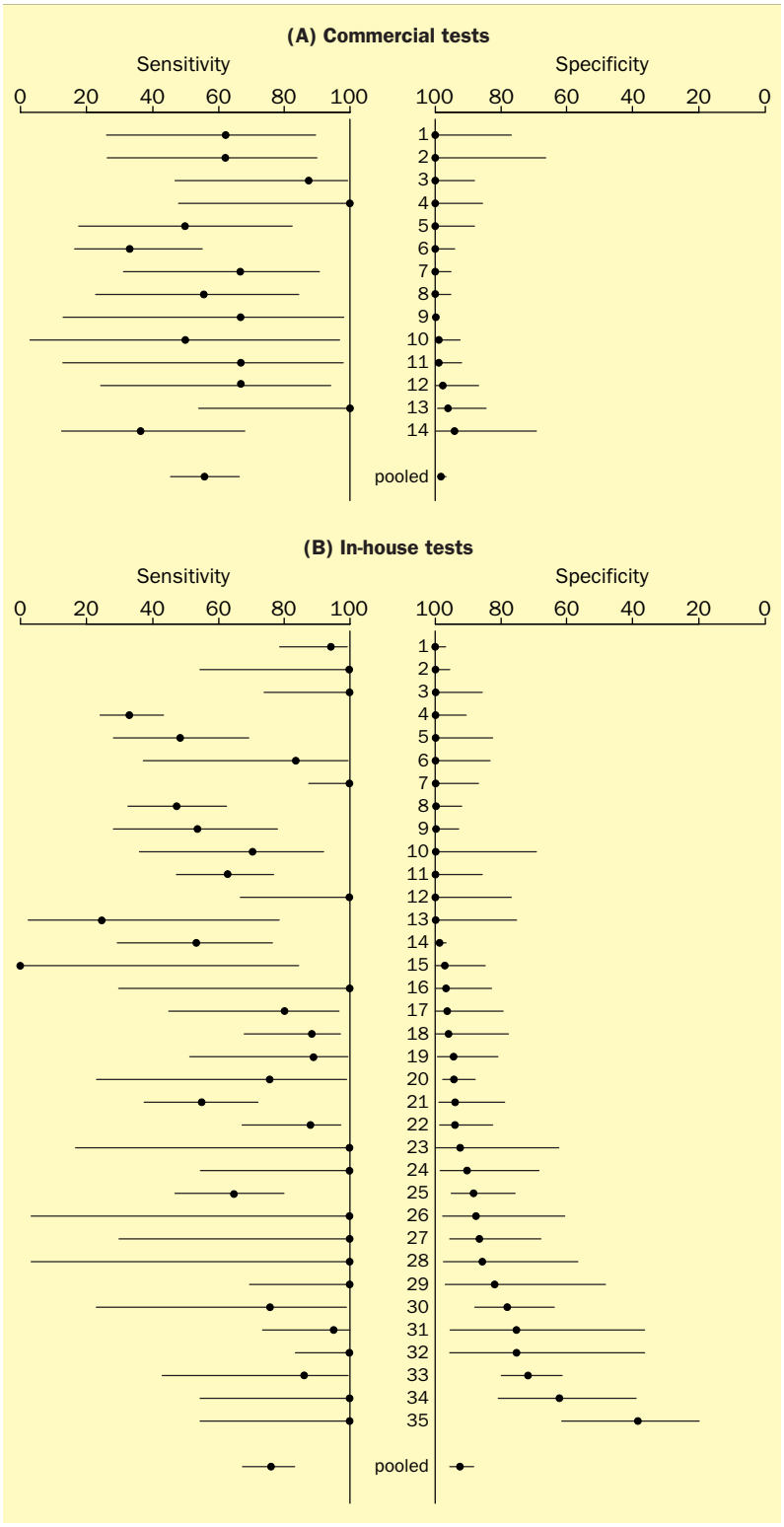
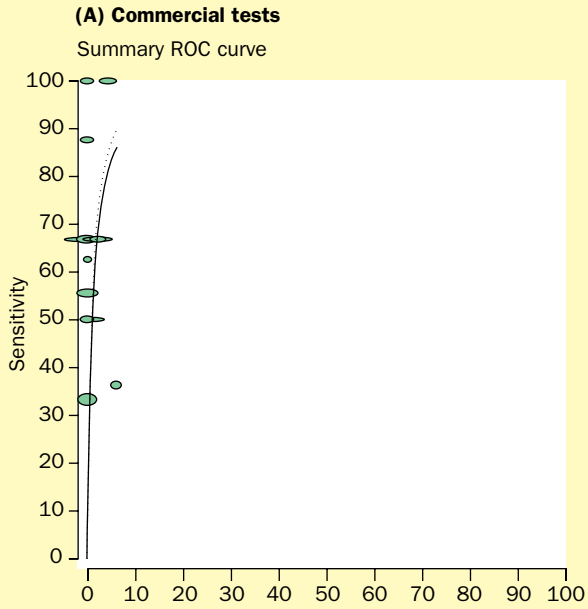


Figure 2. Forest plot of estimates of sensitivity and specificity for commercial and in-house tests. Each solid circle indicates the point estimate of sensitivity and specificity from each study in the meta-analysis. Error bars indicate 95% confidence intervals. Studies are in descending order of specificity. Pooled estimates are summary random effects estimates with 95% confidence intervals. Pooled estimates for commercial tests—sensitivity 0·56 (95% CI, 0·46–0·66), specificity 0·98 (0·97–0·99); in-house tests—sensitivity 0·76 (0·67–0·83), specificity 0·92 (0·88–0·95).



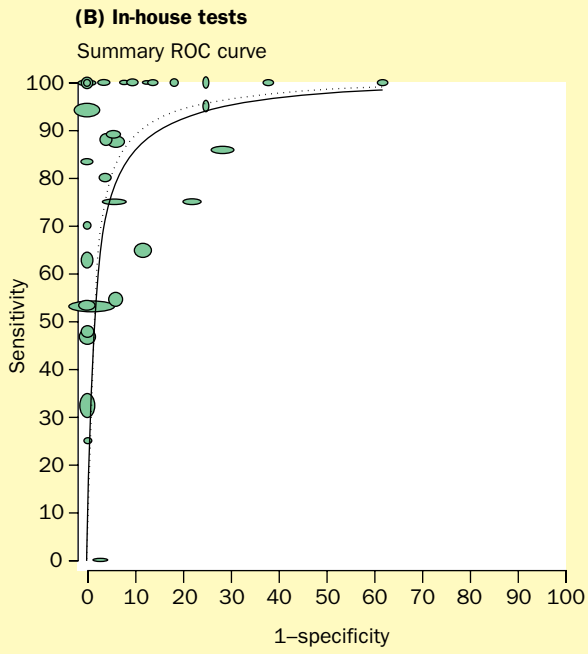


Figure 3. Summary receiver operative (SROC) curves for commercial and in-house tests. Each solid circle represents each study in the meta-analysis. The size of each study is indicated by the size of the solid circle. The weighted (dark line) and unweighted (thin line) regression ROC curves summarise the overall diagnostic accuracy. Area under the curve (unweighted): commercial tests=0·97; in-house tests=0·95.

double blinded. As seen in table 4, even after stratification, heterogeneity persisted in some of the summary estimates. Evaluation of publication bias showed that the Egger test was significant in both the commercial (p=0·01) and in-house tests (p=0·02) subgroups. The funnel plots for publication bias (figure 4) also show some asymmetry. These results indicate a potential for publication bias.

Table 4. Stratified analyses for the evaluation of heterogeneity among studies with in-house tests

Subgroup	Number of studies	Summary diagnostic odds ratio* (95% CI)	Test for heterogeneity† p value
Study design			
Case-control	19	86·5 (39·3, 190·2)	0·03
Cross-sectional	16	43·3 (22·5, 83·3)	0·94
Blinded interpretation of test and/or reference standard results			
Yes	21	46·9 (24·9, 88·6)	0·16
No	14	82·3 (39·8, 170·2)	0·70
Consecutive or random sampling of participants			
Yes	18	63·3 (32·8, 122·4)	0·20
No	17	46·8 (23·6, 92·8)	0·42
Prospective data collection			
Yes	18	59·9 (28·1, 127·6)	0·12
No	17	55·2 (29·9, 101·6)	0·59

*Random effects model. †χ^2 test for heterogeneity. CI=confidence interval.

Discussion

Principal findings

Tuberculous meningitis is a medical emergency. Early and accurate diagnosis is crucial for preventing morbidity and mortality. Our results indicate that commercial tests produce consistent results with high specificity (0·98; 95% CI 0·97, 0·99) and positive likelihood ratios (35·06; 19·03, 64·59). The summary estimate of sensitivity, however, was only 0·56 (0·46, 0·66). Sensitivity estimates were more variable than specificity. Potential explanations for the low sensitivity include a low bacillary load in CSF, testing of inadequate quantity of CSF, and the presence of substances in CSF that inhibit amplification. A positive likelihood ratio of 35 suggests that patients with tuberculous meningitis have a 35-fold higher chance of being NAA test-positive compared with patients without tuberculous meningitis. This ratio suggests a potential role for commercial tests in confirming (ruling in) tuberculous meningitis. However, these tests maximise specificity at the cost of sensitivity, and this trade off has significant clinical implications. By contrast with the high specificity and positive likelihood ratio values, commercial NAA tests have low sensitivity, and negative likelihood ratio values that are not sufficiently low to exclude tuberculous meningitis when a patient's NAA test result is negative. A negative test, therefore, does not mean absence of tuberculous meningitis, and patients with negative NAA results have a fairly high chance of having tuberculosis. The most important finding regarding in-house tests was the substantial variability in the test accuracy.

Clinical implications

As an illustration, consider a patient who is estimated clinically to have a 50% probability of tuberculous meningitis after the initial assessment, and in whom tuberculosis therapy is initiated on clinical grounds, pending culture results. The likelihood that this patient has tuberculous meningitis if the commercial test is positive

increases from 50 to 97%. This high probability would be considered high enough to justify continuation of treatment for tuberculous meningitis. By contrast, if the commercial NAA test result was negative, then the probability that this patient has tuberculous meningitis is 30%, which is not low enough to rule out tuberculous meningitis with great confidence. Our results suggest that a negative NAA test should not be used alone as a justification to discontinue tuberculosis therapy. The decision to discontinue or change treatment should be based on culture results and other relevant clinical data (eg, response to treatment). This finding underscores the need to use NAA tests along with conventional tests such as culture and smear. The clinical implications for institutions that use in-house PCR tests are much less clear. Since the sensitivity and specificity of in-house tests are widely variable, clinicians may have to rely on research data from their own institutions to produce clinically useful estimates of test accuracy.

The accuracy of NAA tests for tuberculous meningitis seems to be similar to those of conventional tests such as culture and smear—high specificity and low sensitivity. This similarity might make NAA tests less useful in practice because they do not have test properties that complement the properties of conventional tests. However, an advantage of NAA tests is the rapidity by which the results can be obtained.[10] In the management of tuberculous meningitis, this rapidity is of great relevance. NAA tests also help to distinguish tuberculous from non-tuberculous mycobacterial (NTM) infections—a feature that might be helpful in populations with high rates of NTM.[10]

NAA tests are not stand-alone tests; they are usually done along with conventional tests. One limitation of our analysis is the lack of data on the incremental gain of using NAA tests over and above the diagnostic performance achieved by use of only conventional methods. The primary studies in our review did not provide such data. Further, the primary studies provided little information on the baseline prevalence of tuberculous meningitis, clinical spectrum of patients, their clinical characteristics, disease severity, co-morbid conditions (such as HIV), and whether the NAA tests were done in patients with low or high clinical suspicion of tuberculosis. There is some evidence that such data might be useful in targeting areas of the clinical spectrum in which NAA tests can contribute to the clinical decision making.[73]

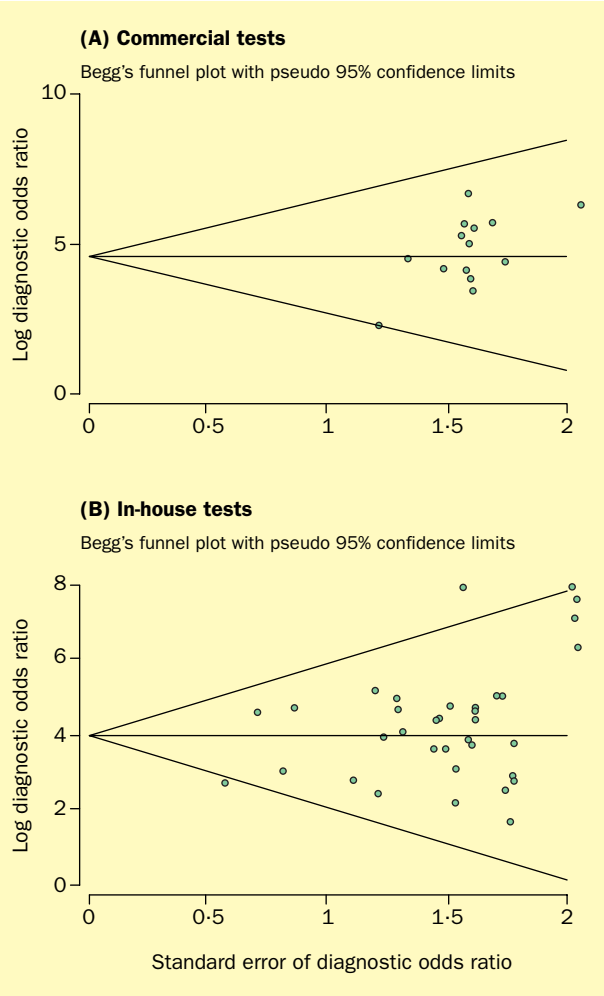


Figure 4. Funnel plots for the assessment of potential publication bias in commercial and in-house test subgroups. The funnel graph plots the log of the diagnostic odds ratio (DOR) against the standard error of the log of the DOR (an indicator of sample size). Each open circle represents each study in the meta-analysis. The line in the centre indicates the summary DOR. In the absence of publication bias, the DOR estimates from smaller studies are expected to be scattered above and below the summary estimate, producing a triangular or funnel shape. The funnel plots appear asymmetric—smaller studies with low DOR estimates are missing—indicating a potential for publication bias. The Egger test for publication bias was significant (p<0·05) in both commercial and in-house test subgroups.

Exploration of heterogeneity

Heterogeneity was a concern in studies that used in-house PCR tests. The shape of the SROC curve suggests that variability in the thresholds used in studies could partly explain the heterogeneity. We also saw that case-control design and lack of blinding were associated with higher test accuracy. This finding is in tune with earlier empiric research which suggests that case-control design and lack of blinding tend to overestimate the true diagnostic accuracy.[16] It is worth noting that considerable unexplained heterogeneity persisted, even after stratification. This may be due to variability in PCR protocols, variability in study setting, and variability in reference standards. For example, in the in-house tests, 15 different methods were used for DNA extraction, and eight different target sequences were amplified. Several large, multicentric studies have assessed the reliability of PCR.[74,75] These studies suggest that PCR tests have poor interlaboratory reliability, and that substantial variability exists in the PCR protocols used by various laboratories.

The effect of study setting is illustrated with the only study in our meta-analysis that had zero sensitivity. Gunisha et al[69] assessed a IS6110-based PCR in Madras, India. The authors attributed the low sensitivity to the fact that, in Madras, earlier research had shown that 40% of *M tuberculosis* isolates had either single or no copies of IS6110,[76] thus explaining such low sensitivity in this study. A key issue in the evaluation of any test for tuberculous meningitis is the lack of a definitive reference standard.[8,9] In general, comparison of a test against

an imperfect reference standard (eg, clinical data without microbiological confirmation) could result in underestimation of test accuracy.[77] In our meta-analysis, exclusion of studies with class three (clinical) reference standards did not have any effect on the summary estimates of sensitivity and specificity among commercial tests.

Strengths and weaknesses of the review

An important strength of our study was its comprehensive search strategy. Screening, study selection, and quality assessment were done independently and reproducibly by two reviewers. Data extraction and quality assessment were done blinded to reduce bias. We reduced the problem of missing data by contacting authors. We also explored heterogeneity and potential publication bias in accordance with published guidelines.[15,24,25]

Our review had some limitations. First, we did not address issues such as cost-effectiveness, reliability, the incremental benefit of adding NAA tests to other tests, and the net effect of NAA tests on clinical care and patient outcomes. Also, because of poor reporting, we could not analyse the effect of factors such as laboratory infrastructure, expertise with NAA technology, patient spectrum, and setting on the accuracy of NAA tests. Second, we could include only English and Spanish language articles due to the linguistic abilities of our team. Thirdly, publication bias was a concern. Exclusion of articles in languages other than English and Spanish may have contributed to this bias. The true accuracy of NAA tests for tuberculous meningitis may be lower than we report.

Our findings should be interpreted in the context of the poor quality of reporting, and variability in study quality. Diagnostic studies in general,[78] and tuberculosis diagnostic studies in particular[79] seem to be beset by these problems. Future developments in NAA testing will need to focus on basic research that will improve test sensitivity as well as strategies to improve study quality and reporting. Our data and previous empiric research[16] suggests that a cross sectional design and use of blinded interpretation of test results might reduce potential bias. Use of guidelines such as the Standards for Reporting of Diagnostic Accuracy[80] (STARD) might improve the quality of reporting. Future studies should also explore the incremental gain of using NAA tests over and above the diagnostic performance achieved by using only conventional tests.

Search strategy and selection criteria

These are described in detail in the Methods section.

Implications

Current evidence suggests a potential role for commercial NAA tests in confirming a diagnosis of tuberculous meningitis. The results of these tests should be interpreted in parallel with clinical findings and the results of conventional tests. Our findings do not support the use of these tests for excluding a diagnosis of tuberculous meningitis. The diagnostic accuracy of in-house PCR tests is poorly defined because of the wide variability in sensitivity and specificity. Clinically useful summary measures cannot be estimated for in-house PCR tests.

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Conflicts of interest

None declared.

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