An assessment of nucleic acid amplification testing for active mycobacterial infection

December 2014

MSAC application no. 1234

Assessment report

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The technical information in this document is used by the Medical Services Advisory Committee (MSAC) to inform its deliberations. MSAC is an independent committee that has been established to provide advice to the Minister for Health on the strength of evidence available on new and existing medical technologies and procedures in terms of their safety, effectiveness and cost-effectiveness. This advice will help to inform government decisions about which medical services should attract funding under Medicare.

MSAC's advice does not necessarily reflect the views of all individuals who participated in the MSAC evaluation.

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Main issues for MSAC consideration

Effectiveness issues

- The two studies that provided direct evidence are likely not generalisable to the Australian setting and the results are probably confounded; therefore, the results are unreliable.
- Culture is an imperfect reference standard; thus, a large proportion of false-positive patients (i.e. NAAT-positive and culture-negative) will likely have clinical disease (see 'Comparison of NAAT and culture, using clinical diagnosis as a reference standard').

Economic issues

- The cost of NAAT is the main driver of the incremental costs in the economic modelling. Large variations in test cost were observed across Australian pathology providers.
- The cost-effectiveness of NAAT is highly sensitive to reductions in the prevalence of TB in the tested population and reductions in the specificity parameters of NAAT.

Financial issues

- The population eligible for NAAT may be an overestimate, as the approach used may include patients who are ineligible for NAAT, such as those suspected of Mycobacterium leprae, patients receiving testing across multiple years for the same infection, or patients who do not have clinical signs and symptoms of a mycobacterial infection.
- As NAAT is currently being used (the extent of which is uncertain), some shifting of costs from the states to the federal health budget is likely but has not been costed in the financial analyses.

Rationale for assessment

Douglass Hanly Moir Pathology Pty Ltd has submitted an application to the Department of Health that requests listing on the Medicare Benefits Schedule (MBS) of nucleic acid amplification testing (NAAT) to diagnose (1) *Mycobacterium tuberculosis* (MTB) infections in persons with clinical signs and symptoms of tuberculosis (TB), or (2) non-tuberculous mycobacteria (NTM) infection in patients suspected of having an NTM infection.

The public funding questions addressed in this contracted assessment of NAAT are largely consistent with the approach pre-specified in the protocol that was ratified by the Protocol

Advisory Subcommittee (PASC). In the evidence collated for this assessment there were no studies with patients who presented with the clinical signs and symptoms of active TB but for whom it was not possible to obtain a specimen suitable for acid-fast bacilli (AFB) microscopy. Therefore, there were no data available to address the clinical management algorithm proposed in Figure 4 (see 'Clinical pathway'), and therefore this clinical indication has not been considered further. It should also be noted that the NTM population eligible for NAAT has been expanded from that specified in the protocol, in order to include all patients suspected of having an NTM infection.

Proposed medical service

NAAT for the detection of active mycobacterial infection is intended to be used in patients suspected of having TB or NTM infections to confirm the presence of the organism and help direct patient management. NAAT to detect MTB will be a separate test from NAAT to detect NTM infections and will require two MBS item numbers.

Nucleic acid amplification test (NAAT)

NAAT can be undertaken using either an in-house (diagnostic laboratory-designed) method or a commercial assay. The methods used for in-house NAAT are usually polymerase chain reaction (PCR)-based, where DNA is amplified via a temperature-mediated DNA polymerase using specific primers complementary to the ends of the targeted sequence. The most widely used commercial NAAT for detection of MTB is the GeneXpert MTB/RIF assay (Xpert, Cepheid, Sunnyvale, CA, USA), which is endorsed by the World Health Organization and has been approved by the Therapeutic Goods Administration (TGA) for use on patient material, regardless of the AFB smear microscopy result. There are no commercially available kits for the detection of NTM approved by the TGA in Australia.

The Guidelines for Australian mycobacteriology laboratories (National Tuberculosis Advisory Committee 2006) state that 'All NAAT methods must be properly validated before routine use'. Commercial tests that have been modified (e.g. for a novel use) and in-house methodologies must be validated according to the *NPAAC guidelines for requirements for the development and use of in-house in-vitro diagnostic medical devices* (National Pathology Accreditation Advisory Council 2014).

Current funding arrangements

Treatment for TB is provided free of charge to patients in Australia. The test to confirm active mycobacterial infection is covered by the state and territory health systems if the patient is a public patient in a public hospital, or by the MBS if the test being performed is

listed on the MBS. The tests currently listed on the MBS are AFB microscopy and culture of suitable specimens. NAAT is not currently listed on the MBS.

Proposal for public funding

An MBS item descriptor was not proposed in the PASC-ratified protocol. Suggested MBS item descriptors, as recommended by the relevant policy area in the Department of Health are provided in Table ES 1.

Table ES 1 Suggested MBS item descriptors

Category 6 – PATHOLOGY SERVICES
MBS item number Nucleic acid amplification test for the detection of *Mycobacterium tuberculosis* complex in patients with signs and symptoms consistent with active tuberculosis. Fee: To be advised
MBS item number Nucleic acid amplification test for the detection of nontuberculous mycobacteria species in patients with a compatible clinical disease. Fee: To be advised

Comparator details

Patients with the clinical signs and symptoms of active TB will receive NAAT in addition to AFB microscopy. Standard microbial testing in Australia for TB, in people with signs and symptoms of active disease, involves AFB microscopy and culture of suitable specimens. As both the intervention and comparator groups receive AFB testing, the main comparator for NAAT is culture alone.

The patient population suspected of having an NTM infection receive NAAT in addition to culture, and this may replace further testing such as additional biopsies. Therefore, the appropriate comparator in the identified population is current testing without NAAT.

Clinical use of the intervention

The use of NAAT in the diagnosis and management of active TB infection is proposed to be an additional diagnostic tool and not a substitute for any of the current tests. NAAT is intended for use with specimens from untreated patients (< 3 days of anti-TB drug treatment) for whom there is clinical suspicion of TB. As the number of bacilli reduces rapidly within days to 2 weeks after commencing appropriate TB treatment (providing the MTB is not drug resistant), MTB cannot be reliably detected in treated patients.

In clinical practice, diagnosis of TB and the selection of an appropriate treatment regimen would be determined by the clinician after taking into account the patient's history and clinical symptoms along with the results of AFB microscopy, NAAT and culture plus drug susceptibility testing (DST). The AFB microscopy and NAAT results would both be available within a day or two, and the interpretation of the AFB and NAAT results would be inter-related. For example, a positive AFB could be the result of an NTM infection rather than MTB, and this would be resolved by the NAAT result.

Key differences in the delivery of the proposed medical service and the main comparator

Currently, most testing for MTB occurs using the AFB smear microscopy and culture tests. Although they are two separate tests, they are usually performed at the same time using the same specimen. The results for the two tests are delivered at different times; AFB microscopy results are reported within 24–48 hours whereas culture results are reported at 6–8 weeks. NAAT would be performed at the same time as AFB microscopy and culture, with results available in the same timeframe as the AFB results.

Clinical claim

Tuberculosis

The applicant proposed that patient outcomes will differ according to the pre-test probability of a patient having TB. Patients with a high pre-test probability of having TB commence antibiotic treatment immediately (i.e. prior to diagnostic confirmation); therefore, NAAT will have limited impact on patient management. In patients at risk of active TB it is proposed that the use of NAAT is non-inferior to current TB testing. However, if NAAT detects rifampicin resistance, its use may cause the treating health professional to change the anti-tubercular drug regimen. This could have public health benefits by reducing the infectiousness of the patient earlier than the 6–8 weeks required for culture and DST. The use of NAAT in this circumstance is proposed to be superior to current testing approaches.

For patients in whom the pre-test probability of TB is low (i.e. AFB-negative and with indeterminate clinical symptoms), the clinical claim is that NAAT is superior to the current standard testing because a positive NAAT would result in immediate treatment that would not have been indicated based on the low pre-test probability of TB.

Non-tuberculous mycobacteria

In patients suspected of having NTM infections, the applicant proposed that NAAT is expected to provide additional diagnostic information to the tests currently performed to

diagnose NTM. The use of NAAT in this population is, therefore, proposed to be superior to the situation where NAAT is not available.

Approach taken to the evidence assessment

A systematic review (SR) of published medical literature was undertaken. Searches to identify relevant studies and reviews for the period between 1990 and June 2014 were conducted for the Cochrane Library, Current Contents, Embase, PubMed, Web of Science, Cinahl, Econlit and Scopus databases, as well as Australian and international health technology assessment (HTA) websites.

For TB infections, studies that investigated the use of NAAT (with or without AFB microscopy) compared with 'no NAAT' (AFB microscopy and culture for diagnostic accuracy studies) in patients suspected of having TB and who have had < 3 days of anti-TB treatment and reported appropriate outcomes, as outlined in Box 1 to Box 4 and Table 24, were included for further review.

For NTM infections, studies that investigated the use of NAAT (with or without AFB microscopy) compared with 'no NAAT' (AFB microscopy and culture or clinical diagnosis) in patients suspected of having an NTM infection were included (see Box 5).

Characteristics of the evidence base

Two studies assessed the direct health impact of NAAT compared with no NAAT on patients suspected of having TB. Seventeen studies reported on the impact of NAAT on the clinical management of patients and 9 studies provided data on the impact of these changes in management on the health outcomes of patients.

Due to the large volume of evidence available on the accuracy of NAAT relative to culture, only studies published after 2005, with 2x2 data suitable for meta-analysis, were included in the final analysis. Studies on the only commercial NAAT product (Xpert) available in Australia were published in 2006 onwards. In-house NAAT, on the other hand, was available before 2005. However, as there have been significant changes in laboratory practice over the past 10 years (Boyle & Pai 2012; Moore, Guzman & Mikhail 2005; Nybo 2012; Public Health and Ambulatory Care 2012), it seemed reasonable to limit study eligibility to publications in the previous decade. A total of 79 studies provided extractable data and were included.

Twelve studies were identified that reported on the diagnostic accuracy of NAAT for the detection of NTM infections and were included in the review. Literature searches spanned the period 1990–2014.

Results of assessment

Safety

No studies were identified that reported on the safety of NAAT compared with current testing. As NAAT is usually conducted on the same samples used for other testing, and there is no need for resampling, no adverse events (AEs) are expected from the testing procedure.

However, more patients will receive a false-positive NAAT than a false-positive AFB result. Therefore, more patients will receive treatment for a disease they do not have and will possibly have an adverse reaction to the anti-TB drugs until clinical unresponsiveness is noted or culture results become available.

Effectiveness of NAAT in the diagnosis of MTB

Direct evidence: does NAAT improve health outcomes?

Both studies assessing the direct health impact of NAAT were conducted in a setting with a high TB prevalence; therefore, the applicability to the Australian healthcare system is questionable. A high-quality randomised controlled trial (RCT) reported no difference in morbidity outcomes at 2 and 6 months follow-up when NAAT and AFB microscopy were compared. However, a strong trend indicating fewer deaths in the NAAT group compared with the AFB microscopy group was observed at 2 months, but this trend was no longer apparent at 6 months. A historical control study of medium quality found no difference in the mortality rate at 2 months follow-up when comparing NAAT with no NAAT. However, both studies were confounded by high levels of treatment initiation based on clinical evidence in the comparator groups.

The difference in treatment initiation between groups in the study by Theron et al. (2014) is unlikely to be reflected in treatment initiation rates in Australia because NAAT is suggested to be used as an adjunct to AFB testing. The incremental impact of NAAT over current testing practice in Australia, and the impact on patient morbidity and mortality, cannot be estimated from this study.

Linked evidence of effectiveness of NAAT in the diagnosis of MTB

Is it accurate?

Meta-analysis of studies investigating the diagnostic accuracy of NAAT compared with culture showed that both in-house NAATs and the commercial Xpert NAAT have diagnostic value for confirming or excluding culture-positive disease. Overall, patients with a positive NAAT result are likely to have culture-positive TB, whereas patients with a negative NAAT result are unlikely to be falsely negative.

In the context of interpreting NAAT results in conjunction with AFB findings, when specimens are AFB-positive a negative NAAT result can confidently exclude the likelihood of an MTB infection (as determined by culture), but a positive NAAT result does not eliminate the possibility of being culture-negative. The explanation for this is that culture is an imperfect reference standard. Culture in AFB-positive specimens likely resulted in misclassification of many of the 22% false-positive results recorded for NAAT.

In AFB-negative specimens a positive NAAT result is likely to correctly confirm the presence of MTB. However, interpretation of a negative NAAT result is dependent on the type of specimen tested. In patients with AFB-negative sputum, a negative NAAT result indicates that the patient may not be culture-positive but it cannot be ruled out. In patients with AFB-negative non-sputum specimens, a negative NAAT result provides no additional useful information. This is likely due to the low numbers of bacilli present in AFB-negative specimens. It should be noted that if few bacilli are present in the specimen, the possibility of a false-negative result would increase for all three tests.

When the results of the included studies were meta-analysed (k=11), NAAT was found to be both highly sensitive (93%, 95%CI 85, 97) and highly specific (98%, 95%CI 96, 99), compared with culture-based DST, in identifying rifampicin-resistant MTB.

Further analyses indicated that there was no difference in the diagnostic accuracy of AFB microscopy or NAAT, compared with culture, in HIV-positive and HIV-negative patients (k=7 and k=6 studies, respectively). As HIV-positive patients commonly produce AFB-negative sputum samples, the difficulty associated with diagnosis of TB in HIV-positive patients is related to the reduced sensitivity of NAAT in this specimen type when compared with AFB-positive specimens.

Does it change patient management?

Not surprisingly, all studies were in agreement that the use of NAAT resulted in a quicker diagnosis of patients with TB, especially in those who were AFB-negative (k=14). Predictably,

this also resulted in earlier treatment in NAAT-positive patients. A historical control study of poor quality and a retrospective cohort study of medium quality reported that the median duration of unnecessary and/or over-treatment of TB was shorter in patients when NAAT was used to guide treatment decisions compared with when NAAT was not available.

There were conflicting data on the likely impact of NAAT in the clinical setting. A retrospective cohort study of poor quality and a high risk of bias, conducted in the UK (medium TB incidence), concluded that clinician decision-making would be affected by NAAT results and that there would be significant clinical benefits from the use of NAAT in low-prevalence settings. Two cohort studies of medium quality, one retrospective and conducted in Saudi Arabia (medium TB incidence) and the other conducted in Canada (low TB incidence), suggest that clinicians would be reluctant to change patient management based on the NAAT result.

Does change in management improve patient outcomes?

Two prospective cohort studies of poor quality, conducted in countries with a low incidence of TB, reported that a delay in time to diagnosis was significantly associated with an increased risk of transmission of TB among contacts. A retrospective cohort study of poor quality, conducted in New Zealand, indicated that, for the individual patient, the time between development of symptoms and diagnosis was not significantly associated with achieving a favourable treatment outcome (i.e. cure or treatment completed).

Three cohort studies (two retrospective) of medium quality provided some evidence that patients with rifampicin-resistant TB who received a rifampicin-containing Category II treatment, before receiving the results of DST had slightly poorer health outcomes than those who did not.

All TB patients are at risk of adverse health events (e.g. hepatitis) associated with first-line treatment. Two SRs, one of medium quality and one of poor quality, found that some, but not all, AEs as a consequence of patients with active TB receiving inappropriate antibiotic treatment (due to MTB resistance) may be avoided with appropriate treatment, to which the MTB strain is sensitive. One SR of good quality found that patients have a higher risk of developing multidrug-resistant TB (MDR-TB) if they receive inappropriate drug treatment.

Overall conclusion with respect to comparative effectiveness

Comparison of AFB, NAAT, and AFB plus NAAT, using culture as the reference standard, showed that AFB plus NAAT (the testing strategy proposed in the application) had the highest false-positive rate of 12%, with NAAT alone at 6% and AFB alone at 2%. A false-

positive result means that a patient will receive treatment for a short time (until clinical unresponsiveness is noted or culture results are available) for a disease they do not have. However, as culture is an imperfect reference standard, a large proportion of these false-positive patients may actually have clinical disease. AFB microscopy alone had the highest false-negative rate at 38%, whereas NAAT alone or AFB plus NAAT were much lower at 11% and 6%, respectively. The consequences of a false-negative result are much more severe, as the patient may remain untreated for a longer time period and could potentially spread the disease to more individuals in the community.

The results of the meta-analyses presented in this report suggest that NAAT would be a useful addition to AFB microscopy and culture in the diagnosis of both pulmonary and extrapulmonary TB. Patients with a positive AFB test or NAAT result are likely to have culture-positive TB, and it becomes almost certain if both tests are positive. No useful information can be obtained directly from a negative AFB result, as these patients may or may not have TB. A negative NAAT result should be interpreted with reference to the AFB result—in a patient who was AFB-positive it almost completely eliminates the likelihood of being MTB culture-positive; conversely, in a patient who was AFB-negative it does not eliminate the possibility of culture-positive disease.

The use of NAAT enables quicker diagnosis and treatment of patients with TB, especially in those who are NAAT-positive and AFB-negative. It also reduces the duration of unnecessary and/or over-treatment for TB, particularly in those patients who are NAAT-negative and AFB-positive.

The accuracy of NAAT compared with culture-based DST indicates that NAAT can accurately identify patients with rifampicin-resistant MTB. Thus, NAAT could be used to inform the type of antibacterial treatment offered to TB patients. This would help avoid side effects such as hepatitis from inappropriate use of rifampicin, and earlier appropriate treatment for rifampicin resistance would also reduce the risk of developing MDR-TB.

Linked evidence of diagnostic effectiveness of NAAT in the diagnosis of NTM

NAAT to detect NTM could be separated into three distinct categories: detecting NTMs in general (NTM-NAAT), specifically detecting *M. avian* complex (MAC) strains (MAC-NAAT), and detecting *M. ulcerans* in patients suspected of having Buruli ulcer. The pooled accuracy of MAC-NAAT compared with culture showed that patients with a positive MAC-NAAT result were most likely to be infected with *M. avian*, but it is equivocal whether patients with a negative result have a culture-positive MAC infection (k=5 studies). Patients with a positive NTM-NAAT were more likely to have an infection than not, and patients with a negative

result were more likely to be uninfected with NTM than to be infected (k=5 studies). The area under the summary receiver–operator characteristic (SROC) curve indicated that both NTM- and MAC-NAAT performed well in predicting culture positivity. There was insufficient evidence of the accuracy of NAAT in the diagnosis of NTM in AFB-positive or -negative specimens, so no conclusions could be reached about the value of NAAT in conjunction with AFB microscopy in the detection of NTM infections.

It should be noted that culture is an imperfect reference standard. When compared with a clinical reference standard, the median sensitivity for NTM-NAAT (k=2) was higher than for culture or AFB microscopy. NAAT appears to be able to identify a larger proportion of patients with an NTM infection than either AFB microscopy or culture. The results of these meta-analyses should be viewed with caution due to the small number of studies included and the wide 95% confidence intervals (CIs) for many of the analyses.

Other relevant considerations

TB in the Indigenous population

The incidence of TB in the Australian Indigenous population was 11 times higher than in the Australian non-Indigenous population in 2010[1]. Higher rates of hospitalisation and mortality from TB also occur in the Indigenous population. The rapid diagnosis and treatment of TB is essential in remote communities in order to quickly contain the spread of infection. This is particularly important for children and infants, given the challenges in accessing adequate health care in these communities. Point-of-care testing with same-day results would likely offer easier access to diagnosis and more rapid treatment initiation in small regional hospitals and clinics in rural areas of Australia, if suitable training of personnel was available.

Xpert is the first fully automated NAAT developed for the point-of-care diagnosis of MTB and rifampicin-resistant MTB, and was endorsed by the World Health Organization (WHO) in December 2010 (WHO 2014). Three studies that met the inclusion criteria looked at the use of Xpert in a point-of-care setting. One study reported that nurse-administered Xpert results had substantial agreement with those done by a laboratory technician on paired sputum specimens (κ=0·69, 95%CI 0·64, 0·74), and a similar sensitivity and proportion of unusable results. Two studies reported that most patients who were Xpert-positive were started on anti-TB treatment on the same day as specimen collection, compared with a median delay of 13–14 days for Xpert-negative patients.

[1] Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

In addition, the early knowledge of rifampicin resistance may influence treatment decisions, ensuring that appropriate anti-TB drugs are given immediately, thus reducing the likelihood of developing MDR-TB.

Economic evaluation

A cost–utility analysis is presented to assess the cost-effectiveness of adding NAAT to AFB smear microscopy, and culture and sensitivity (C&S), testing in a population with clinical signs and symptoms of active TB. A summary of the structure of the mechanics of the economic model is presented in Table ES 2.

Table ES 2 Summary of the economic evaluation

Time horizon	20 months
Outcomes	Quality-adjusted life-years (QALYs)
Costs	Australian dollars, 2014 prices
Methods used to generate results	Decision tree analysis
Discount rate	5% costs and outcomes accrued beyond 1 year
Software packages used	Microsoft Excel

QALY = quality-adjusted life-year

As clinical management in Australia differs depending on the clinical suspicion (pre-test probability) of TB, the model is separated into patients with:

- a high clinical suspicion of TB, where treatment is initiated based on clinical suspicion, and the benefit of NAAT is to identify resistance mutations and initiate appropriate earlier treatment for MDR; and
- a low clinical suspicion of TB, where treatment decisions are initiated or delayed based on AFB ± NAAT results. In addition to earlier MDR treatment initiation, additional benefits of NAAT include the ability to differentiate between patients with MTB and NTM infections (who would have previously been treated on the basis of the AFB results alone), and to reduce the delay in treatment in those with true TB who returned a negative AFB result (who would not have been treated without the availability of NAAT).

Additional scenarios are presented to examine the extent to which treatment initiation decisions based on clinical suspicion affect the cost-effectiveness of NAAT.

Key model assumptions

- When AFB and NAAT are discordant, the treatment decision is based on NAAT (i.e. consistent with PASC protocol).
- C&S testing (the reference standard) is assumed to be 100% sensitive and specific, as all patients have C&S testing and at the end of 2 months all will have a correct diagnosis (i.e. MDR-TB, TB or no TB).

- Once the decision to initiate or delay treatment has been made, the model assumes that there will be no change in treatment until the results of C&S are available. This assumption may favour NAAT, as the earlier initiation of resistant drugs in the comparator arm would reduce the benefit of introducing NAAT.
- Cost and utility penalties associated with the secondary transmission of TB are applied for each index case in the model, but the consequences (i.e. cost or health outcome) of further ongoing transmissions (e.g. tertiary transmissions and beyond) are not included in the base-case.

The incremental cost-effectiveness of NAAT is presented, incorporating costs in a stepped manner. The base-case incremental cost-effectiveness ratio (ICER) for NAAT is $90,728/ QALY. The addition of NAAT leads to more patients initially receiving the correct treatment, due to improved sensitivity in conjunction with AFB and the ability of NAAT to identify MDR-TB. The incremental cost of NAAT is driven predominantly by the cost of testing, offset by reduced TB transmissions and hospitalisation costs. The incremental QALY gain is driven by the shift in patients from being initially untreated TB (or standard treatment in the case of MDR-TB) to receiving correct treatment.

Table ES 3 The incremental cost-effectiveness of NAAT

Utilities considered	Costs included (NAAT cost applied in AFB + NAAT arm)	ICER
Index patient utility	Treatment only	$188,307
	Treatment and AEs	$188,238
	Treatment, AEs and management	$185,882
	Treatment, AEs, management and hospitalisation	$145,956
	Treatment, AEs, management, hospitalisation and transmission	$103,978
Index and secondary case utility	Treatment, AEs, management, hospitalisation and transmission	$90,728

AEs = adverse events; ICER = incremental cost-effectiveness ratio

Sensitivity analyses for the base-case (TB mixed) scenario were conducted around a number of parameters included in the economic modelling (using 95%CI or plausible upper and lower limits). The ICER is most sensitive to changes in the prevalence of TB in the tested population (decreasing the prevalence from 22% to 10% in the tested population increases the ICER to $967,000) and the specificity of NAAT (ICERs exceeding $200,000 when the lower limit of NAAT specificity estimates are used).

Financial implications

A market-based approach is taken, using MBS data to estimate the number of patients who utilised at least one item of mycobacterial AFB microscopy, culture and sensitivity (MC&S) testing in 2009–13, and to project the expected number of patients who would be eligible for NAAT for TB and NTM (as requested) in 2015–19. One NAAT is assumed per eligible

patient. However, as this assumption may underestimate the number of tests when multiple mycobacteria are suspected (i.e. TB may be initially suspected with a pulmonary infection, but if negative then NAAT may be used to test for *M. kansasii* and/or MAC). It is unclear how often this situation would occur—the applicant has estimated this in approximately 30% of patients initially suspected of TB.

As NAAT is not intended to replace current testing, the estimated net financial implication to the MBS is equal to the cost of the requested NAAT listings multiplied by the expected number of services. The financial implications to the MBS resulting from the proposed listings of NAAT are summarised in Table ES 4.

Table ES 4 Financial implications of proposed NAAT listings

	2015	2016	2017	2018	2019
Projected number of patients eligible for NAAT	37,575	39,299	41,022	42,745	44,468
Population suspected of TB					
Proportion of patients suspected of TB	50%	50%	50%	50%	50%
Number of patients suspected of TB	18,788	19,650	20,511	21,373	22,234
Proposed NAAT fee	$130.00	$130.00	$130.00	$130.00	$130.00
Proportion of patients bulk-billed	61%	61%	61%	61%	61%
MBS fees associated with TB listing	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
MBS benefits payable (85%)	$2,076,074	$2,171,325	$2,266,466	$2,361,717	$2,456,857
Patient co-payments [a]	$144,715	$151,354	$157,986	$164,626	$171,257
Population suspected of NTM					
Proportion of patients suspected of NTM	50%	50%	50%	50%	50%
Number of patients suspected of NTM	18,788	19,650	20,511	21,373	22,234
Proportion of initial TB suspects tested	30%	30%	30%	30%	30%
Number of initial TB suspects tested	5,636	5,895	6,153	6,412	6,670
Total number of patients tested for NTM	24,424	25,545	26,664	27,785	28,904
Proposed NAAT fee	$50.00	$50.00	$50.00	$50.00	$50.00
Proportion of patients bulk-billed	61%	61%	61%	61%	61%
MBS fees associated with NTM listing	$1,221,220	$1,277,250	$1,333,215	$1,389,245	$1,445,210
MBS benefits payable (85%)	$1,038,037	$1,085,663	$1,133,233	$1,180,858	$1,228,429
Patient co-payments [a]	$72,357	$75,677	$78,993	$82,313	$85,629
MBS fees associated with NAAT listings	$3,663,660	$3,831,750	$3,999,645	$4,167,735	$4,335,630
MBS benefits payable (85%)	$3,114,111	$3,256,988	$3,399,698	$3,542,575	$3,685,286
Patient co-payments [a]	$217,072	$227,031	$236,979	$246,938	$256,886

[a] Only payable by patients who are not bulk-billed
NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; TB = tuberculosis

The approach used may overestimate the population eligible for NAAT, as testing of patients suspected of *M. leprae* may be included (but would not be eligible for NAAT) and, as these tests are used to monitor treatment effectiveness, patients may receive testing across

multiple years for the same infection. Furthermore, the current MBS items are not restricted to patients with clinical signs and symptoms of a mycobacterial infection; as testing may be ordered as part of the initial work-up of a chronic obstructive pulmonary disease or some renal diseases, this approach may further overestimate the eligible population.

Given the uncertainties in estimating the eligible population, the financial implications of introducing NAAT are uncertain. However, as NAAT is proposed to be used as an add-on test, net costs to the MBS are implied. Estimates presented in the assessment ($3.7—$4.3 million over the 5-year period) are likely to represent the upper limits of proposed use, as all assumptions regarding the eligible population are likely to be overestimated. The financial implications are most sensitive to changes in the cost per test. While benefits associated with reduced transmissions may be expected, these have not been quantified.

As NAAT is currently being used (the extent of which is uncertain), some shifting of costs from the states to the federal health budget is anticipated, and so the net societal cost of NAAT may be lower than the net costs to the MBS.

Glossary and abbreviations

Abbreviation	Definition
AE	adverse event
AFB	acid-fast bacilli
AHTA	Adelaide Health Technology Assessment
ARTG	Australian Register of Therapeutic Goods
AUC	area under the curve
CI	confidence interval
CSF	cerebrospinal fluid
C&S	culture and sensitivity
DST	drug susceptibility testing
FNA	fine-needle aspirate
HESP	Health Expert Standing Panel
HIV	human immunodeficiency virus
HTA	health technology assessment
ICER	incremental cost-effectiveness ratio
IVD	in-vitro diagnostic
KPS	Karnofsky performance score
LAMP	loop-mediated isothermal amplification
LR+	positive likelihood ratio
LR–	negative likelihood ratio
MAC	*Mycobacterium avium* complex
MBS	Medicare Benefits Schedule
MDR	multidrug resistant/resistance
MDR-TB	multidrug-resistant tuberculosis
MC&S	AFB microscopy, culture and sensitivity
MSAC	Medical Services Advisory Committee
MTB	*Mycobacterium tuberculosis*
NAAT	nucleic acid amplification test(ing)
NHMRC	National Health and Medical Research Council

Abbreviation	Definition
NTM	non-tuberculous mycobacteria
PASC	Protocol Advisory Subcommittee (of MSAC)
PBS	Pharmaceutical Benefits Schedule
PCR	polymerase chain reaction
QALY	quality-adjusted life-year
QoL	quality of life
RCT	randomised controlled trial
SR	systematic review
SROC	summary receiver–operator characteristic
TB	tuberculosis
TGA	Therapeutic Goods Administration
ZN	Ziehl-Neelsen

Introduction

This assessment report is intended for the Medical Services Advisory Committee (MSAC). MSAC evaluates new and existing health technologies and procedures for which funding is sought under the Medicare Benefits Schedule (MBS) in terms of their safety, effectiveness and cost-effectiveness, while taking into account other issues such as access and equity. MSAC adopts an evidence-based approach to its assessments based on reviews of the scientific literature and other information sources, including clinical expertise.

Adelaide Health Technology Assessment (AHTA), School of Population Health, University of Adelaide, was commissioned by the Australian Government Department of Health to conduct a systematic literature review and economic evaluation of the nucleic acid amplification test (NAAT) in the diagnosis of active mycobacterial infection. This evaluation has been undertaken in order to inform MSAC's decision-making regarding public funding of NAAT.

The proposed use of NAAT for active mycobacterial infection in Australian clinical practice was outlined in a protocol that guided the evaluation undertaken by AHTA. The protocol was released for public comment in March 2014. No public consultation responses were received. The protocol was finalised as a result of PASC deliberations at a meeting on 12–13 December 2013.

Rationale for assessment

Douglass Hanly Moir Pathology Pty Ltd submitted an application to the Department of Health to create new MBS item(s) for NAAT to diagnose: (1) *Mycobacterium tuberculosis* (MTB) infections in persons with clinical signs and symptoms of tuberculosis (TB) or (2) non-tuberculous mycobacteria (NTM) infection in patients who have tissue biopsies with histopathology consistent with an NTM infection.

It should be noted that the NTM population eligible for NAAT has been expanded from the population specified in the protocol, in order to include all patients suspected of having an NTM infection. The expanded population base was necessary due to the the insufficient evidence-base for NTM infections as a whole. There was also value in including information on patients with specimen types other than tissue biopsies, such as HIV-positive patients presenting with *M. avium* complex (MAC) disease or patients with disseminated bacteraemia.

Background

Tuberculosis

Tuberculosis (TB) is an infectious disease caused by the bacterial genus *Mycobacterium*. The majority of disease is caused by MTB-complex species (including *M. tuberculosis, M. africanum, M. bovis, M. microti, M. canettii, M. caprae, M. pinnipedii* and *M. mungi*). However, disease caused by NTM, such as *M. avium*, *M. kansasii*, *M. xenopi* and *M. malmoense,* also occurs. It is a major global health problem; in 2012 an estimated 8.6 million people developed TB and 1.3 million died from the disease, including 320,000 deaths among human immunodeficiency virus (HIV)-positive people (WHO 2013). Even though Australia has a low rate of TB, with 4.7–6.5 cases per 100,000 population in 2010–12 (Lumb et al. 2013; WHO 2013), the total number of TB cases increased by 33% between 1998 and 2008, with most new cases occurring in arrivals from countries where TB is endemic (National Tuberculosis Advisory Committee 2012).

In Australia TB is a notifiable disease.National guidelines have been developed on the public health management of this disease (CDNA 2013). TB continues to pose ongoing challenges due to an increasing incidence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant strains. A major concern articulated in the Australian Government TB policy is the entry into Australia of individuals infected with drug-resistant TB from Papua New Guinea via the Torres Strait (Marais, Sorrell & Britton 2012).

TB is transmitted through respiratory droplets from persons with active pulmonary or laryngeal TB. In rare cases invasion of MTB may occur through mucous membranes or damaged skin. It most commonly affects the lungs but may affect almost any organ or system, including the lymph nodes, central nervous system, liver, bones, genitourinary tract, and gastrointestinal tract (Cruz-Knight & Blake-Gumbs 2013; Garcia-Monco 2014). Extrapulmonary TB occurs in 10–42% of patients, depending on their ethnic background, age and immune status, as well as the presence or absence of underlying disease and the genotype of the MTB strain (Zumla et al. 2013). Table 1 lists the clinical symptoms associated with the classic presentation of TB.

Table 1 Clinical presentations of TB

Site of infection	Clinical symptoms
Pleural TB	Blood-tinged sputum producing chronic cough, pleurisy, chest pain.
TB lymphadenitis	Enlarged cervical or supraclavicular lymph nodes.
Tuberculous meningitis	Persistent or intermittent headache for 2–3 weeks; mental status changes, coma.

Site of infection	Clinical symptoms
Head and neck TB	The presenting complaints can include lump in the neck, nasal obstruction, sore throat or discomfort, external nasal lesions and otitis media.
Skeletal TB	Clinical presentation includes localised pain associated with fever and weight loss. Spine is most common site (Pott disease). Back pain, stiffness, lower extremity paralysis (50%).
Tuberculous arthritis	Involves the joints. Hips and knees more commonly affected. Pain precedes radiographic changes.
Cutaneous TB	Lesions show a wide spectrum of morphology including tuberculous chancre, TB verrucosa cutis, lupus vulgaris, scrofuloderma, orificial TB, miliary TB, metastatic TB abscess, and most cases of papulonecrotic tuberculid.
Pericardial TB	Clinical features include cough, weight loss, fever, night sweats and anorexia.
Genitourinary TB	The kidneys are the most common site of infection causing flank pain, dysuria and frequent urination. Men may present with a painful scrotal mass, prostatitis, orchitis or epididymitis. In women the condition may mimic pelvic inflammatory disease. Causes 10% of sterility in women worldwide and 1% of women in industrialised countries.
Renal TB	Renal TB is usually a complication of a previous primary pulmonary infection. MTB form cortical granulomas, and on reactivation spread into the medulla, causing papillitis. Advanced disease leads to cortical scarring, and infundibular and pelvic strictures. The end result of diffuse disease is destruction, loss of function and calcification of the entire kidney.
Gastrointestinal TB	TB may infect any site along the gastrointestinal tract. TB can manifest as non-healing ulcers of the mouth or anus, difficulty swallowing, abdominal pain (e.g. peptic ulcer), malabsorption, painful diarrhoea or haematochezia. Can also affect the liver, spleen and pancreas.
Ocular TB	Ocular TB can affect nearly every ocular tissue. Clinical manifestations include vitritis, macular oedema, retinal periphlebitis, choroiditis uveitis, retinal vasculitis and serpiginous-like choroiditis.
TB in the breast	Breast TB is rare and can present as clinical suspicion of carcinoma due to the development of granulomas; it can also present as mastitis. At later stages it erodes through the skin, causing ulceration and discharging sinus tracts.
TB from joint replacement surgery	MTB prosthetic joint infection is most often caused by reactivation of prior tuberculous arthritis.

Sources: Abbara & Davidson (2011); Abes, Abes & Jamir (2011); Al-Mezaine et al. (2008); Al-Serhani (2001); Bani-Hani et al. (2005); Berbari et al. (1998); Cruz-Knight & Blake-Gumbs (2013); Kakkar et al. (2000); Muttarak, ChiangMai & Lojanapiwat (2005); Reuter et al. (2006)

Some people have a high risk of infection due to an increased likelihood of exposure to an infected individual (CDNA 2013), such as:

- new arrivals and recently returned travellers from countries with a high TB incidence
- contacts of an active case within the past 5 years
- people living in overcrowded conditions, such as some Indigenous Australians in localised areas (e.g. Northern Territory, Queensland) or in institutions
- healthcare workers who serve or have served high-risk populations.

The fate of the mycobacteria in a newly infected individual is dependent on the person's immune system. A healthy immune system may clear the bacterium or, alternatively, exposure can lead to latent TB or progress to primary active TB (Cruz-Knight & Blake-Gumbs 2013). Most infections in humans are asymptomatic and latent, and can persist for a lifetime. In the healthy host, progression to active TB occurs in approximately 10% of those

infected. For half of these patients this progression occurs within 2 years, and in the other half it can occur up to decades later (CDNA 2013; Zumla et al. 2013). Once infected, some patients are more susceptible to progression to active TB than others (CDNA 2013). These include:

- children younger than 5 years of age, adolescents and the elderly
- people who are malnourished
- people who are immunocompromised due to:
  - diseases such as HIV, diabetes and renal failure
  - immunomodulating therapies, such as corticosteroids, anti-TNF inhibitors and anti-cancer treatments.

Patients with a respiratory infection that is unresponsive to standard treatment should be suspected of having TB if they belong to one of these high-risk populations. Standardised TB treatment for an appropriate period of time will cure over 98% of drug-sensitive cases (HKCS/BMRC 1987). Deaths from TB in Australia are usually due to co-morbidities or delays in diagnosis and treatment (CDNA 2013). The success of treatment and the prevention of drug resistance and relapse relies heavily on the compliance of the healthcare provider in prescribing the right drug combination, dose and duration of treatment, as well as on patient adherence to treatment.

The aim of government policy is to prioritise screening of higher risk groups such as Aboriginal and Torres Strait Islander peoples and persons born overseas (including immigrants, students, healthcare workers), engage in regional TB control programs and ensure that there is a high standard of diagnosis and treatment (National Tuberculosis Advisory Committee 2012).

Drug-resistant mycobacterial infections

MDR-TB[2] and extensively drug-resistant TB[3] are serious global public health problems (Abubakar et al. 2013). In Australia MDR-TB occurs in 2–3% of cases and extensively drug-resistant TB is uncommon (Lumb et al. 2013). Treatment of drug-resistant TB is difficult to manage, requires a long duration, requires the use of drugs that are less potent and more toxic, and may result in poor health outcomes.

[2] Defined as resistant to rifampicin and isoniazid

[3] Defined as resistant to rifampicin, isoniazid, fluoroquinolones, and any of the second-line injectable drugs such as capreomycin, amikacin and kanamycin

Resistance to anti-TB drugs is the result of spontaneous mutations in the genome of MTB and is caused by inappropriate monotherapy and intermittent treatment with anti-TB drugs (Abubakar et al. 2013; Lemos & Matos 2013). Resistance occurs at rates that are predictable for each drug, varying from 1 in every 10^{2-4} bacilli for pyrazinamide to 1 in every 10^{7-8} bacilli for rifampicin (Lemos & Matos 2013).

Combination treatments can successfully prevent the emergence of resistance during the treatment of TB. Any MTB that becomes resistant to one drug can be killed by the other drug and vice versa (Lemos & Matos 2013; Mitchison 2012).

Non-tuberculous mycobacterial infections

Non-tuberculous mycobacteria (NTM) are environmental mycobacteria, and do not include the MTB pathogens or *M. leprae* that causes Hansen's disease or leprosy (Runyon 1959). Disease caused by NTM is not notifiable in Australia; hence, there is little information on the incidence or prevalence of NTM disease. Clinically significant pulmonary and extrapulmonary NTM cases represent approximately one-third of all NTM pulmonary isolates and two-thirds of all extrapulmonary isolates processed by laboratories in Queensland (Thomson 2010; Thomson et al. 2013).

Table 2 lists the *Mycobacterium* species isolated in Queensland in 2005 and the proportion of pulmonary or extrapulmonary disease that was caused by each species. Of the isolates from pulmonary sites, most of the clinically significant disease was caused by *M. intracellulare*, *M. avium* and *M. kansasii*; whereas for non-pulmonary sites, most clinically significant disease was caused by *M. fortuitum*, *M. abscessus, M. chelonae, M. intracellulare*, *M. peregrinum* and *M. avium*.

Table 2 Proportion of mycobacterial isolates causing clinically significant and non-significant pulmonary and extrapulmonary disease in Queensland, 2005

Mycobacteria species	Significant pulmonary	Not significant pulmonary	Significant extrapulmonary	Not significant extrapulmonary
M. intracellulare	16.2%	20.1%	4.9%	3.5%
M. avium	3.4%	5.7%	4.2%	0.7%
M. kansasii	2.0%	1.2%	–	–
M. abscessus	1.4%	3.4%	6.3%	1.4%
M. chelonae	0.6%	1.8%	5.6%	2.1%
M. scrofulaceum	0.6%	1.4%	2.1%	0.7%
M. gordonae	0.4%	3.3%	0.7%	1.4%
M. fortuitum	0.2%	3.5%	16.1%	4.9%
M. peregrinum	–	0.4%	4.9%	–
M. ulcerans	–	–	2.8%	–
M. haemophilum	–	0.2%	0.7%	1.4%

Mycobacteria species	Significant pulmonary	Not significant pulmonary	Significant extrapulmonary	Not significant extrapulmonary
M. smegmatis	–	–	0.7%	
M. szulgai	–	–	0.7%	–
M. lentiflavum	–	1.0%	–	0.7%
M. asiaticum	–	0.6%	–	0.7%
M. simiae	–	0.4%	–	–
M. mucogenicum	–	0.4%	–	0.7%
M. nonchromogenicum	–	0.2%	–	–
M. marinum	–	–	–	0.7%
M. asiaticum	–	–	–	0.7%

Numbers in **bold** highlight the three most common pulmonary and extrapulmonary NTM species that were responsible for significant disease in 2005.
Source: Thomson (2010)

The incidence of pulmonary disease due to NTM has been increasing worldwide. Some of the reasons for this increase include greater awareness of NTM as pulmonary pathogens, the introduction of new technologies and improvements in existing methods, enabling better detection and more-accurate identification of NTM isolates. In addition, NTM is more prevalent in an ageing population.

NTM organisms originate from environmental sources such as food, other animals, soil or water. Pulmonary NTM infections are the most common and are usually caused by the MAC group. *M. kansasii*, *M. xenopi* and *M. malmoense* are the next most common causes, with their prevalence varying among American and European countries (Borchardt & Rolston 2013; Martin-Casabona et al. 2004).

Skin and soft-tissue NTM infections, often originating from a cut or graze, manifest clinically as rashes, ulcers, nodules, granulomas, cellulitis or abscesses. NTM skeletal infections of bones, joints and tendons primarily occur following accidental trauma, surgery, puncture wounds or injections. These infections can be localised or multifocal, and can progress to septic arthritis, osteomyelitis and even bacteraemia. Disseminated NTM infections are almost exclusively limited to severely immunocompromised persons (Borchardt & Rolston 2013).

Nucleic acid amplification test (NAAT) for active mycobacterial infection

In-house NAAT

Most in-house NAAT methods are polymerase chain reaction (PCR)-based. The PCR process amplifies DNA via a temperature-mediated DNA polymerase, using specific primers that are complementary to the ends of the targeted sequence. PCR is carried out with a series of alternating temperature steps or cycles: (1) 92–95 °C to denature the DNA so that the two

strands separate, (2) a lower temperature, usually between 45 °C and 60 °C, to allow annealing of the primer sequences to the single-stranded DNA and (3) an amplification step at the optimal temperature for the DNA polymerase, usually 65 °C. PCR can be used to amplify targeted gene sequences that vary in length from 100 bases to over 20,000 bases. For the detection of DNA sequences specifically associated with MTB or NTM, the targeted sequence is usually small, around 100 bases, but may be as large as 500 bases.

A commonly occurring problem with PCR is that primers can bind to incorrect regions of the DNA, for example to a related gene from another bacterial species, resulting in unexpected non-specific products. Several modified PCR methods are used to overcome this problem.

Nested PCR involves two sets of primers used in two successive runs of PCR; the second set amplifies a secondary smaller target region within the first PCR product. Thus, the second region is only amplified if the first product was amplified from the intended target sequence and not from a non-specific sequence.

Real-time PCR is a quantitative method where the amplified product is detected as the reaction progresses. This method often uses fluorescent dyes to detect the PCR product. The number of cycles required and the quantity obtained of the product can be used to determine if the amplified product is due to the specific target. Products that require additional cycles and are slow to amplify are often non-specific.

Reverse transcription is used to detect and amplify RNA sequences using an enzyme called reverse transcriptase, which transcribes the RNA of interest into its DNA complement. Subsequently, the newly synthesised complementary DNA is amplified using traditional PCR. Reverse-transcription PCR can be combined with quantitative real-time PCR for quantification of RNA.

Multiplex PCR consists of multiple primer sets within a single PCR mixture to produce products of varying sizes that are specific to different DNA sequences. By targeting multiple genes at once, additional information may be gained from a single test run. Thus, one PCR run could be used to both identify MTB using an MTB-specific target and detect the presence of specific mutations that confer antibiotic resistance, such as the well-documented mutation in the *rpoB* gene that confers rifampicin resistance.

Loop-mediated isothermal amplification (LAMP) is an isothermal non-PCR-based amplification method in which isothermal amplification is carried out at a constant temperature. This method employs a DNA polymerase and four to six specially designed

primers that recognise a total of six to eight distinct sequences on the target DNA (Figure 1a).

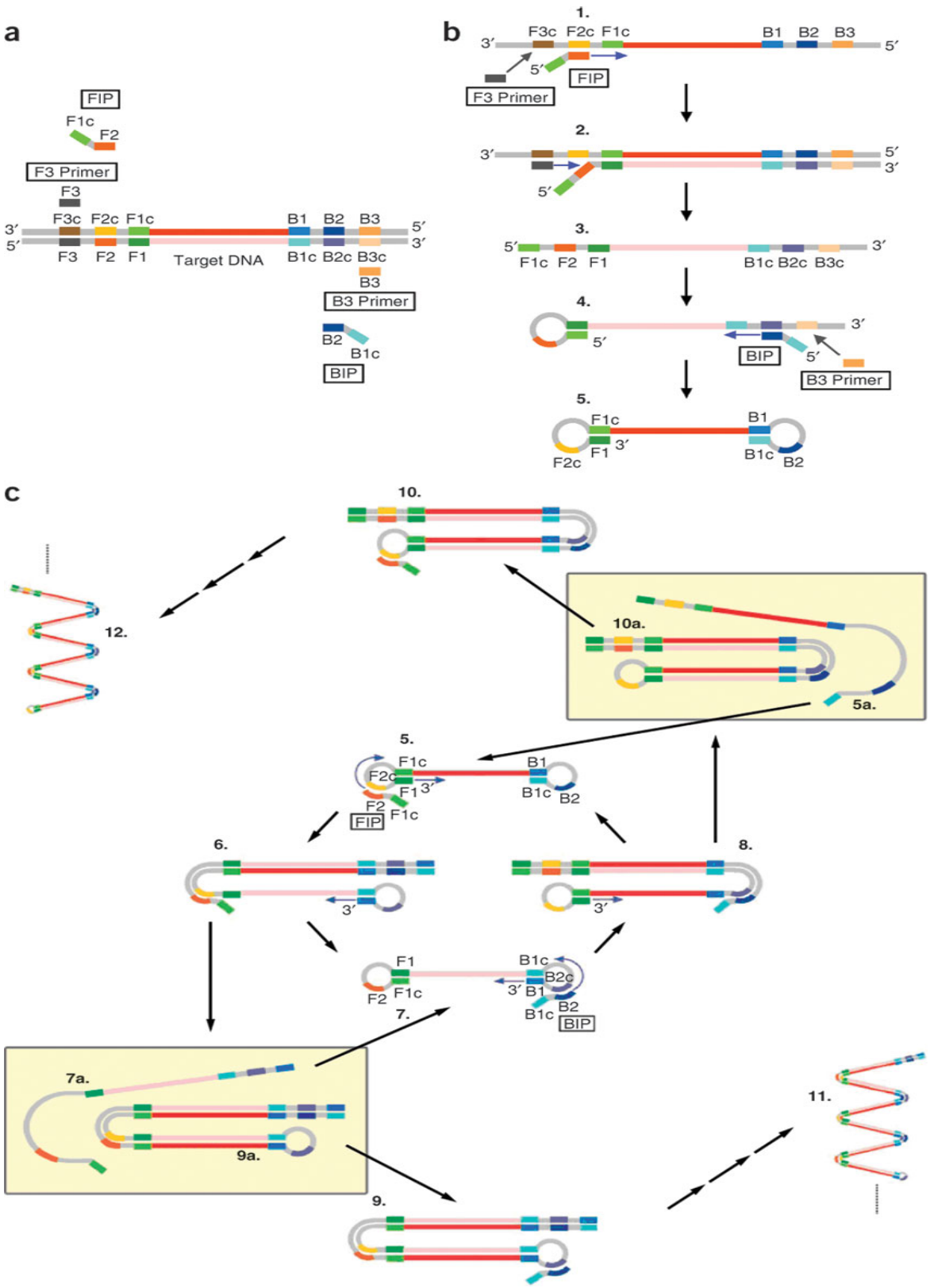

Figure 1 Principles of the LAMP method

(a) Primer design of the LAMP reaction (b) Starting structure producing step (c) Cycling amplification step

Source: Tomita et al. (2008)

LAMP is then initiated by the binding of an inner primer containing sequences of the sense and antisense strands of the target DNA. Strand displacement DNA synthesis is primed by an outer primer causing the release of a single-stranded DNA, which serves as a template for DNA synthesis that is primed by a second primer pair that hybridise to the end of the target to produce a stem–loop DNA structure (Figure 1b). In subsequent LAMP cycling, one inner primer hybridises to the loop on the product and initiates the displacement DNA synthesis (Figure 1c). This results in the accumulation of 10^9 copies of the target in less than an hour. LAMP is relatively new and less versatile than PCR, and the primer design is much more difficult than for PCR, requiring computer programs as it is subject to numerous constraints (Torres et al. 2011). LAMP may also be combined with a reverse transcription step to allow the detection of RNA.

Commercial NAAT

The most widely used commercial NAAT for detection of MTB is the GeneXpert MTB/RIF assay (Xpert, Cepheid, Sunnyvale, CA, USA), which is endorsed by the World Health Organization (WHO) and has been approved by the TGA for use on patient material, regardless of the acid-fast bacilli (AFB) smear microscopy result.

The Xpert assay is a semi-quantitative, nested real-time PCR test that uses a cartridge containing all elements necessary for the reaction (Association of Public Health Laboratories 2013; Lawn et al. 2013). The Xpert assay detects MTB and rifampicin resistance (considered to be a reliable proxy for MDR-TB) in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either AFB microscopy positive or negative. The Xpert assay system simplifies molecular testing by fully integrating and automating sample preparation, real-time PCR amplification and detection using a six-colour laser (Association of Public Health Laboratories 2013).

The assay simultaneously detects MTB-complex and the genetic mutations associated with rifampicin resistance by amplifying an MTB-complex-specific 81-bp sequence from the core region of the *rpoB* gene. The assay is based on this region as it accounts for 95% of all known rifampicin-resistant mutations in MTB, and all known mutations in this region confer rifampicin resistance (El-Hajj et al. 2001; Lawn et al. 2013). It then uses five differently coloured fluorogenic nucleic acid probes, which fluoresce only when bound to their target sequence. Each probe is highly specific and binds to a different segment of the amplified core region, as shown in Figure 2. If the amplified sequence differs from the target rifampicin-susceptible sequence by as little as a single nucleotide substitution, the probe will not bind (El-Hajj et al. 2001). The assay also includes a sample-processing control probe,

which will fluoresce even if the assay cannot detect any MTB in the sample, to distinguish between a true negative result and test failure (El-Hajj et al. 2001).

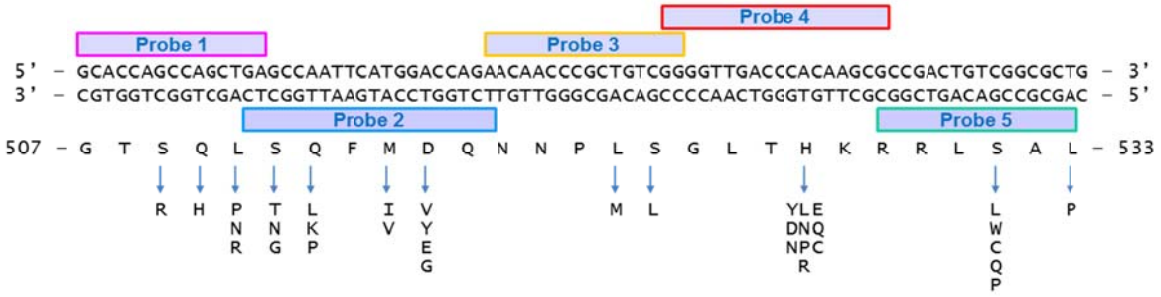

Figure 2 The 81-bp MTB-specific rifampicin-resistance determining region of the *rpoB* gene

The hybridisation sites of the five Xpert fluorogenic probes are shown. The single letter codes for the amino acids encoded by this region and the common single amino acid substitutions that confer rifampicin resistance are also shown. Changes in codon Ser531 and His526 account for more than 70% of the mutations in this region.

Sources: Adapted from Cepheid Xpert MTB/RIF brochure (Cepheid), Casali et al. (2014) and Rattan et al. (1998)

Thus, the interpretation of the Xpert NAAT results (assuming the sample-processing control probe is positive, indicating that the test has not failed) is as follows:

- negative for MTB if one or no probes are fluorescent
- positive for MTB if at least two of the five probes are fluorescent
- rifampicin-resistant MTB detected if two to four probes are fluorescent
- rifampicin-resistant MTB not detected if all five probes are fluorescent.

There are no commercially available kits for the detection of NTM listed on the Australian Register of Therapeutic Goods (ARTG). However, there are nine listed by the US Food and Drug Administration; three of these kits detect *M. avium*, one kit each detect *M. kansasii, M. gordonae, M. intracellulare*, and three kits are rapid diagnostic systems for mycobacteria[4].

Recently, NAAT has also been used for diagnosis of TB from extrapulmonary specimens (Lawn et al. 2013).

Intended purpose

NAAT is intended for use with specimens from untreated patients (i.e. < 3 days of anti-TB drug treatment) for whom there is a clinical suspicion of TB. As the number of bacilli reduces rapidly within days to 2 weeks after commencing appropriate TB treatment (providing the MTB is not drug resistant), MTB cannot be reliably detected in treated patients.

[4] US Food and Drug Administration, 'Medical devices, nucleic acid based tests'. Available from URL: http://www.fda.gov/medicaldevices/productsandmedicalprocedures/invitrodiagnostics/ucm330711.htm (accessed 12 June 2014)

The applicant recommended that NAAT should only be performed in institutions proficient in the culture and identification of MTB. Transport and storage at 2–8 °C is important for this test, and samples should preferably be read within 24–48 hours (prolonged storage > 4 days has been reported to impact on results). Results can be provided to clinicians within 24–48 hours.

There are recognised guidelines for Australian mycobacteriology laboratories that specify the biosafety procedures, infrastructure, equipment and work practices required by the laboratory (National Tuberculosis Advisory Committee 2006). Laboratories performing TB cultures must participate in a recognised quality assurance program.

Clinical need

The use of NAAT in the diagnosis and management of active TB infection is proposed to be an addition to the current clinical algorithm and does not substitute for any current test.

Both the Australian National Tuberculosis Advisory Committee (National Tuberculosis Advisory Committee 2006) and the Association of Public Health Laboratories in the USA (Association of Public Health Laboratories 2013) strongly recommend that all specimens received for NAAT also undergo both AFB microscopy (where possible) and culture and drug susceptibility testing (DST). This would occur regardless of the NAAT result confirming the presence or absence of MTB.

The rationale for this recommendation relates to the view that knowledge of the AFB microscopy result, in conjunction with a NAAT result, can better inform clinical decisions. For example, a NAAT-negative, AFB-positive specimen in conjunction with patient history and clinical presentation could contribute to ruling out MTB infection, and may suggest an NTM infection. Patients with HIV and pulmonary TB have a higher likelihood of being AFB microscopy negative (de Albuquerque et al. 2014; Scherer et al. 2011), so a NAAT-positive result could be useful for managing TB in these patients.

Culturing the organism is still important, as a negative NAAT does not exclude the possibility of a positive culture. Additionally, a positive NAAT does not differentiate among the species of MTB or determine the presence of other *Mycobacterium* species (Association of Public Health Laboratories 2013).

As the Xpert assay only determines the presence or absence of rifampicin resistance, all MTB isolates should receive additional DST using culture-based methods to determine the susceptibility patterns of other first- and second-line drugs used to treat TB (Association of Public Health Laboratories 2013).

The applicant has proposed that patient outcomes will differ according to the pre-test probability of a patient having TB. Given the public health implications of active pulmonary TB, patients with a high pre-test probability of having TB (approximately 20% of those tested, of whom 50–70% will actually have TB) commence antibiotic treatment immediately. Of the remaining 80% of patients with a low pre-test probability of having TB and in whom treatment is delayed until culture results are available, only 5–10% will actually have TB. In this population the applicant has suggested that the use of NAAT is non-inferior to current practice.

When rifampicin-resistant MTB is detected by NAAT in patients who have already started treatment, clinicians are provided with information on whether the patient's treatment is likely to be effective within a few days, and this can lead to a change in case management. There are theoretical public health benefits associated with reducing the infectiousness of the patient earlier. Currently, a change in the antibiotic regimen would be due to ongoing AFB tests (where they can be collected), indicating that a patient is either not responding to treatment or is waiting for the result of the culture and DST in 6–8 weeks. In this situation the applicant has suggested that the use of NAAT may be superior to current practice.

For patients whose pre-test probability of TB is low, the applicant has suggested that positive NAAT results would result in immediate treatment that would not normally have been indicated, given the patient's TB risk assessment.

In patients suspected of having an NTM infection, NAAT is expected to be an additional test to those currently performed to diagnose NTM.

Existing tests for diagnosing *Mycobacterium* species

NAAT for mycobacteria is currently not listed on the MBS. However, some Australian diagnostic laboratories, such as Alfred Health[5] and PathWest Pathology Services[6], offer in-house NAAT (MTB PCR) for screening specimens from patients with suspected TB.

Currently, most testing for MTB occurs using both AFB smear microscopy and culture tests. Although they are two separate tests, they are usually performed at the same time using the same specimen. The results for these two tests are delivered at different times; AFB

[5] Alfred Health Pathology Service, *Mycobacterium tuberculosis* PCR. Available from URL: http://pathology.alfred.org.au/handbook/ (accessed 19 June 2014)

[6] PathWest Laboratory Medicine WA, *Mycobacterium tuberculosis* PCR. Available from URL: http://www.pathwest.com.au/testdirectory/ (accessed 19 June 2014)

microscopy results are reported within 24–48 hours, whereas culture results are reported at 6–8 weeks.

AFB smear microscopy involves spreading a suitable specimen thinly onto a glass slide, treating it with an acid-fast stain (Ziehl-Neelsen (ZN), Kinyoun stain or auramine-rhodamine stain) and examining the stained slide under a microscope (Lab Tests Online 2012). Results are typically available between several hours and 1 day after a sample is collected. AFB microscopy is ordered when:

- the patient has symptoms that suggest pulmonary or extrapulmonary TB
- the patient has a positive TB screening test and is at increased risk for active disease and/or has characteristic lung involvement as shown by X-ray
- an individual has been in close contact with a person who has been diagnosed with TB and has either symptoms or a condition that increases their risk of contracting the disease
- for monitoring purposes during treatment for TB
- an immunosuppressed patient is systemically unwell and they are screened for unusual infections such as mycobacteria and fungi.

Cultures are used to diagnose active MTB and NTB infections, to help determine whether the TB is confined to the lungs or has spread to other organs, to monitor the effectiveness of treatment, and to help determine when a patient is no longer infectious (Lab Tests Online 2012). Traditionally, cultures have used semi-solid agar-based media and require 4–8 weeks for sufficient growth to obtain a diagnosis. However, newer liquid culture systems are approximately 10% more sensitive for detection of mycobacteria than semi-solid media, and can obtain results in days rather than weeks (WHO 2007). One drawback is that liquid culture is more prone to contamination with other microorganisms (WHO 2007).

DST is usually conducted in conjunction with a culture to determine the most effective antibiotics to treat the infection. The mycobacteria are grown in the presence of anti-TB drugs, either in liquid or semi-solid media, and compared with growth when the drug is absent. If growth of the MTB is detected in the presence of the anti-TB drug, it indicates drug resistance (TBFacts.org). Liquid culture systems can reduce the delay for results to as little as 10 days instead of several weeks (WHO 2007).

Marketing status of device

NAAT for the detection of mycobacteria may be an in-house assay or a commercial kit. In December 2010 the WHO endorsed the Xpert assay for the rapid and accurate detection of

MTB and rifampicin-resistant MTB. This test was approved by the TGA in April 2013 and by the U.S. Food and Drug Administration in July 2013.

Summary of TGA approval[7] for the IVD Class 3 GeneXpert MTB/RIF assay:

ARTG entry number: 207732

Sponsor: Cepheid Holdings Pty Ltd

Intended purpose: The GeneXpert MTB/RIF assay for use with the Cepheid GeneXpert system is a semi-quantitative, nested real-time PCR in-vitro diagnostic (IVD) test for the detection of:

- MTB-complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either AFB smear positive or negative
- rifampicin-resistance associated mutations of the *rpoB* gene in samples from patients at risk for rifampicin resistance

The GeneXpert MTB/RIF assay is intended for use with specimens from untreated patients for whom there is clinical suspicion of TB.

No other commercially available NAATs for the detection of MTB and/or NTM are approved by the TGA.

An in-house NAAT for the detection of MTB is classified as a Class 3 IVD medical device by the TGA. IVDs are pathology tests and related instrumentation used to carry out testing on human samples, where the results are intended to assist in clinical diagnosis or in making decisions concerning clinical management. From 1 July 2014 all IVDs must comply with a set of essential principles for their quality, safety and performance. Laboratories that manufacture Classes 1–3 in-house IVD medical devices must comply with the requirements of Part 6A, Schedule 3, of the Regulations (Therapeutic Goods Administration 2011).

To meet these requirements the laboratory must be accredited as a medical testing laboratory by either the National Association of Testing Authorities or a conformity assessment body determined suitable by the TGA, and meet the National Pathology Accreditation Advisory Council (National Pathology Accreditation Advisory Council 2014) performance standard requirements for the development and use of in-house IVDs (Therapeutic Goods Administration 2012). The Guidelines for Australian mycobacteriology

[7] Therapeutic Goods Administration, Australian Register of Therapeutic Goods (ARTG). Available from URL: https://www.ebs.tga.gov.au/ (accessed 19 June 2014)

laboratories (National Tuberculosis Advisory Committee 2006) also state that these requirements must be met.

Current reimbursement arrangements

Treatment for TB is provided free of charge to patients in Australia. Testing to confirm active mycobacterial infection is only covered if the patient is a public patient in a public hospital or if the test performed is listed on the MBS. Standard microbial testing for TB in people with signs and symptoms of active disease in Australia involves AFB microscopy and culture of suitable specimens, and these tests are listed on the MBS (Table 3).

Table 3 Current MBS item descriptors for diagnosing active mycobacterial infections

Category 6 – PATHOLOGY SERVICES
69324 Microscopy (with appropriate stains) and culture for mycobacteria - 1 specimen of sputum, urine, or other body fluid or 1 operative or biopsy specimen, including (if performed): (a) microscopy and culture of other bacterial pathogens isolated as a result of this procedure; or (b) pathogen identification and antibiotic susceptibility testing; including a service mentioned in item 69300 Fee: $43.00 Benefit: 75% = $32.25 85% = $36.55
69325 A test described in item 69324 if rendered by a receiving approved pathology practitioner (Item is subject to rule 18) Fee: $43.00 Benefit: 75% = $32.25 85% = $36.55
69327 Microscopy (with appropriate stains) and culture for mycobacteria - 2 specimens of sputum, urine, or other body fluid or 2 operative or biopsy specimens, including (if performed): (a) microscopy and culture of other bacterial pathogens isolated as a result of this procedure; or (b) pathogen identification and antibiotic susceptibility testing; including a service mentioned in item 69300 Fee: $85.00 Benefit: 75% = $63.75 85% = $72.25
69328 A test described in item 69327 if rendered by a receiving approved pathology practitioner (Item is subject to rule 18) Fee: $85.00 Benefit: 75% = $63.75 85% = $72.25
69330 Microscopy (with appropriate stains) and culture for mycobacteria - 3 specimens of sputum, urine, or other body fluid or 3 operative or biopsy specimens, including (if performed): (a) microscopy and culture of other bacterial pathogens isolated as a result of this procedure; or (b) pathogen identification and antibiotic susceptibility testing; including a service mentioned in item 69300 Fee: $128.00 Benefit: 75% = $96.00 85% = $108.80

69331 A test described in item 69330 if rendered by a receiving approved pathology practitioner (Item is subject to rule 18) Fee: $128.00 Benefit: 75% = $96.00 85% = $108.80

Source: MBS Online. Available from URL: http://www.health.gov.au/internet/mbsonline/publishing.nsf/Content/Downloads-201407 (accessed 16 June 2014)

Proposal for public funding

The application did not provide a proposed MBS item descriptor.

Patients with signs and symptoms of active MTB, and patients suspected of having an NTM infection, are two different populations that require different NAATs and different MBS item descriptors. Suggested MBS item descriptors are listed in Table 4.

Table 4 Suggested MBS item descriptors

Category 6 – PATHOLOGY SERVICES
MBS item number Nucleic acid amplification test for the detection of *Mycobacterium tuberculosis* complex in patients with signs and symptoms consistent with active tuberculosis. Fee: To be advised
MBS item number Nucleic acid amplification test for the detection of non-tuberculous mycobacteria species in patients with a compatible clinical disease. Fee: To be advised

NAAT to diagnose MTB infections should be conducted on both AFB microscopy positive and negative specimens and on all pulmonary and extrapulmonary specimen types.

NAAT to diagnose NTM infections should be able to detect the most common NTM species associated with pulmonary and extrapulmonary disease, as determined by the state Mycobacterium Reference Laboratories.

NAAT to diagnose NTM infections is intended to be conducted in tissues with granulomatous change in both AFB-positive and -negative specimens, where MTB is not a consideration or has been excluded by an MTB-specific NAAT.

PASC advice is that there should be no limit on the number of tests per year per patient in the MBS item descriptor.

There are a number of NAATs currently listed on the MBS. These range from detection of microbial nucleic acid (item 69494), with a Medicare fee of $28.85, to the amplification and determination of hepatitis C virus genotype (item 69491), with a Medicare fee of $206.20. The application reports that the New South Wales state reference laboratory charges $200 for TB PCR, which is billed to the patient. During the assessment NAAT costs in Australia were found to vary substantially, from $28.65 to $130 or perhaps more if confirmation testing is required. The Victorian reference laboratory[8] indicated that an in-house NAAT costs $82 and the commercial Xpert kit $130. In this instance the costs are met primarily through the Victorian State Government (only private patients and non-Australian residents are billed for testing).

Consumer impact statement

No consumer responses were received during the public consultation period.

[8] Personal communication, received 1 october 2014

Approach to assessment

Objective

To determine whether there is sufficient evidence, in relation to safety, effectiveness and cost-effectiveness, to have NAAT listed on the MBS for the diagnosis of MTB in patients with the signs and symptoms of active TB, and NTM in patients suspected of having an NTM infection.

A systematic review (SR) of published medical literature was undertaken. Searches to identify relevant studies and reviews for the period between 1990 and June 2014 were conducted for the Cochrane Library, Current Contents, Embase, PubMed, Web of Science, Cinahl, Econlit and Scopus databases, as well as Australian and international health technology assessment (HTA) websites.

Clinical pathway

The clinical management algorithms for patients with the signs and symptoms of active TB are shown in Figure 3 and Figure 4. The pathways underwent public consultation and incorporated both expert opinion and guidance from the Centers for Disease Control[9]. There is a view by experts that the major factor in the current clinical management of a patient with the signs and symptoms of active TB is the patient's pre-test probability of having TB.

Figure 3 presents current and proposed clinical management algorithms for patients with the clinical signs and symptoms of active TB and for whom AFB microscopy can be done; that is, the patient can provide a sample for testing. This includes samples such as sputum, bronchoalveolar lavage, bronchial aspirates, gastric aspirates and stool for the diagnosis of pulmonary TB; and samples such as cerebrospinal fluid (CSF), urine, lymph node fine-needle aspirates (FNAs) or any other body fluid or tissue sample for the diagnosis of extrapulmonary TB. Currently, clinicians rely on the results of AFB microscopy, as well as whether the patient has a high or low pre-test probability that they will have active TB, as the basis to initiate or defer antibiotic treatment. NAAT is suggested as an adjunctive test that would be performed concurrently with AFB microscopy and culture.

[9] Centers for Disease Control and Prevention. URL: http://www.cdc.gov/ (accessed during writing of the protocol)

Figure 4 presents current and proposed clinical management algorithms for patients who present with the clinical signs and symptoms of active TB and from whom it is not possible to obtain a specimen suitable for AFB microscopy. The only patients that could be identified to fit this profile were those for whom it was not possible to obtain a sample for any purpose. Due to the lack of a sample, the effectiveness of NAAT is not assessible in these patients. Thus, this algorithm could not be addressed in the assessment and will not be discussed further.

Figure 5 presents current and proposed clinical management algorithms for patients who are suspected of having an NTM infection. It should be noted that the NTM population eligible for NAAT has been expanded from the population specified in the protocol, in order to include patients presenting with specimens other than tissue biopsies, such as sputum specimens to be tested for MAC disease or blood samples for disseminated NTM. It should also be noted that histology is not able to differentiate between MTB and NTM infections and it is assumed that culture will also be performed. The expanded population base was necessary due to the insufficient evidence-base for NTM infections as a whole. The proposed use of NAAT will substitute for current testing.

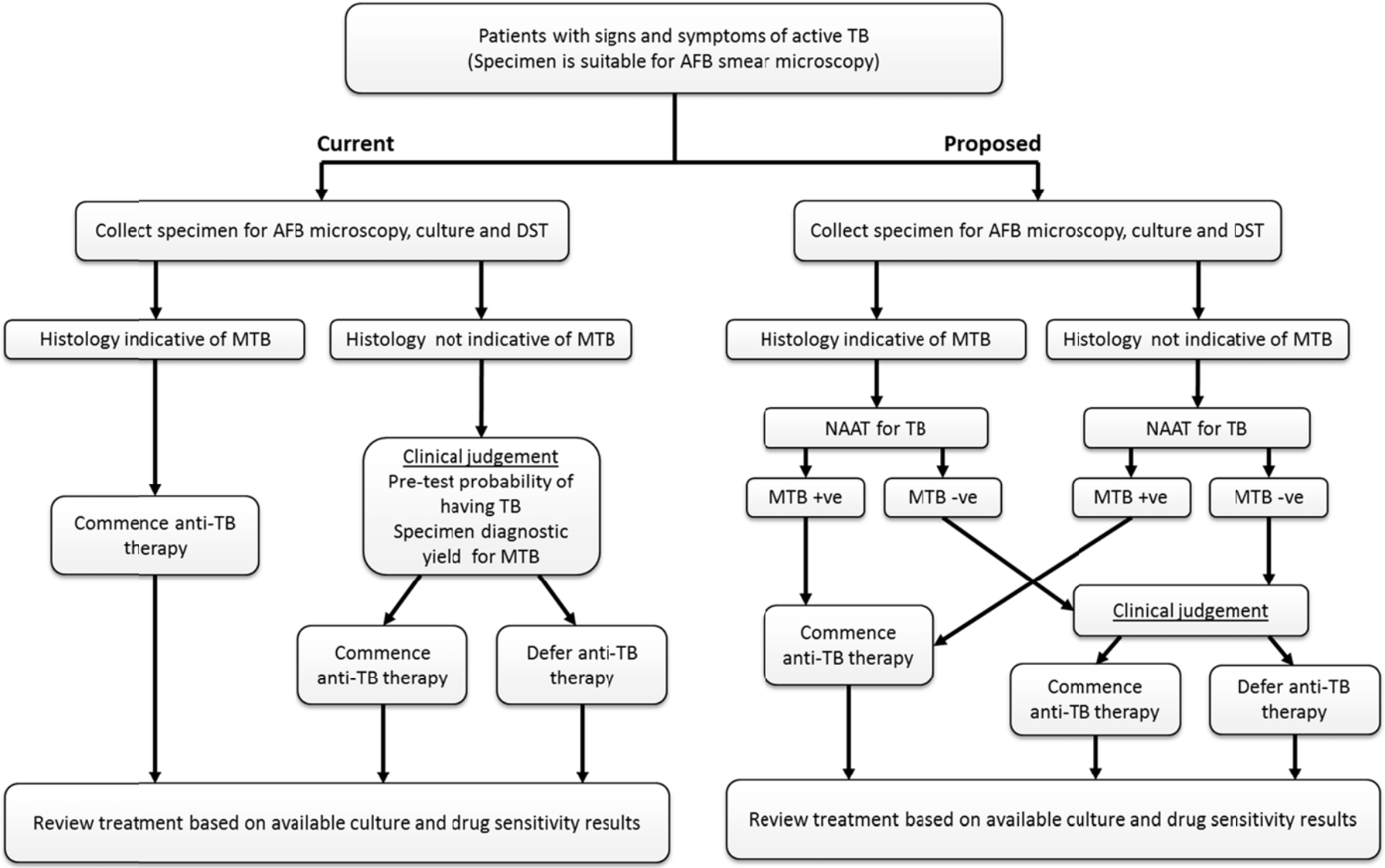

Figure 3 Current clinical management of TB and proposed use of NAAT for active TB where AFB is obtained

AFB = acid-fast bacilli; DST = drug susceptibility testing; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification testing; TB = tuberculosis

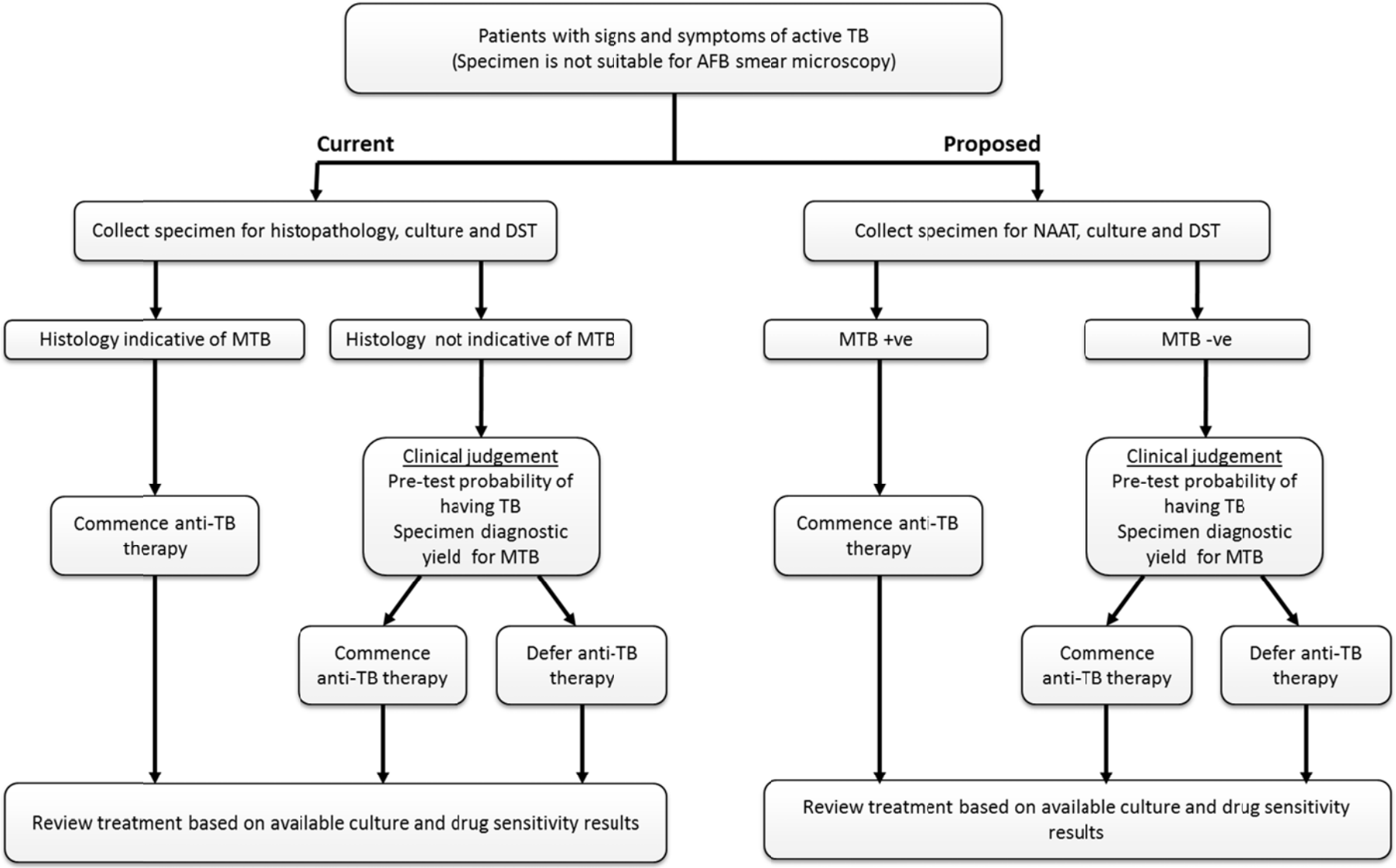

Figure 4 Current clinical management and proposed algorithm with use of NAAT for active TB where AFB microscopy is not able to be obtained

AFB = acid-fast bacilli; DST = drug susceptibility testing; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification testing; TB = tuberculosis

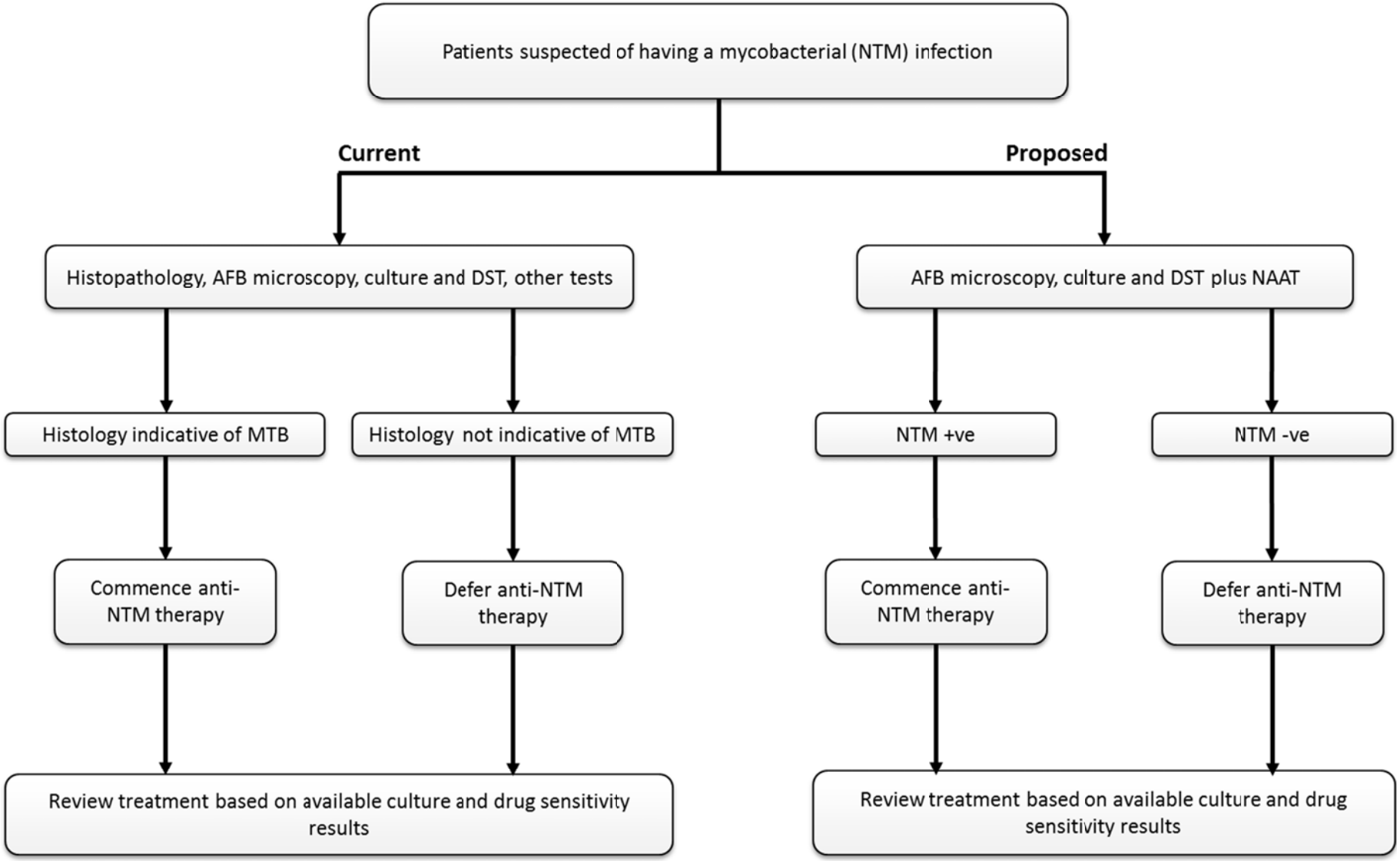

Figure 5 Current clinical management algorithm and proposed algorithm with use of NAAT for patients who are suspected of having an NTM infection

AFB = acid-fast bacilli; DST = drug susceptibility testing; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification testing

Comparator

Patients with the clinical signs and symptoms of active TB will receive NAAT in addition to AFB microscopy. Standard microbial testing in Australia for TB, in people with signs and symptoms of active disease, involves AFB microscopy and culture of suitable specimens. As both the intervention and comparator groups receive AFB testing, the main comparator for NAAT is culture alone.

The patient population suspected of having an NTM infection receive NAAT in addition to culture, and this may replace further testing such as additional biopsies. Therefore, the appropriate comparator in the identified population is current testing without NAAT.

The reference standard

The accuracy of NAAT at determining the presence of MTB or NTM in a specimen will be determined using culture as the reference standard. The diagnostic accuracy of NAAT in determining the presence of rifampicin-resistant MTB in a specimen will also be determined using DST as the reference standard.

It is important to note that culture is an imperfect reference standard for the diagnosis of MTB and NTM. Not all patients who are clinically diagnosed as having TB or NTM infections (on the basis of histopathology, symptoms and response to drug therapy, as well as culture) will have received a positive culture result.

Research questions

Outlined below are the clinical questions formulated according to the information provided in the protocol, which was revised and accepted by the Protocol Advisory Subcommittee (PASC) of the MSAC.

Research questions:

- What are the safety, effectiveness, and cost-effectiveness of NAAT versus NAAT plus AFB microscopy in diagnosing TB in patients who have signs and symptoms of TB?
- What are the safety, effectiveness, and cost-effectiveness of NAAT versus current testing in diagnosing NTM in patients suspected of having an NTM infection?

Subquestions (for a linked evidence approach):

Accuracy

- What is the accuracy of NAAT in the diagnosis of patients with suspected MTB, compared with AFB microscopy and culture?
- What is the accuracy of NAAT plus AFB microscopy in the diagnosis of patients with suspected MTB, compared with AFB microscopy alone?
- What is the accuracy of in-house NAAT compared with commercial NAAT in the diagnosis of patients with suspected MTB, using culture as the reference standard?
- What is the accuracy of NAAT in the detection of genetic mutations on the *rpoB* gene that are associated with rifampicin resistance?
- What is the accuracy of NAAT in the diagnosis of NTM in patients suspected of having an NTM infection, compared with culture?

Change in management

- Does AFB microscopy plus NAAT to determine the presence of MTB and rifampicin resistance change patient management, compared with management decisions made based on AFB microscopy alone, in patients with a high pre-test probability of active TB?
- Does AFB microscopy plus NAAT to determine the presence of MTB and rifampicin resistance change patient management, compared with management decisions made based on AFB microscopy alone, in patients with a low-pre-test probability of TB?
- Does NAAT plus culture change patient management, compared with culture plus other tests, in patients suspected of having an NTM infection?

Effectiveness of change in management

- To what extent does treating patients who have rifampicin-resistant MTB infections with alternative treatments result in better health outcomes for the patient and their contacts?
- What is the health impact of early versus delayed treatment of TB on the individual and their contacts?
- What adverse events (AEs) are associated with inappropriate antibiotic treatment for TB?

Diagnostic assessment framework

This assessment uses the theoretical framework outlined in the MSAC *Guidelines for the assessment of diagnostic technologies* (MSAC 2005).

This means that evidence of the clinical effectiveness of diagnosing MTB or NTB using NAAT requires either:

- evidence of the effectiveness of NAAT from high-quality comparative studies evaluating the use of NAAT and subsequent treatment, compared with culture plus DST and treatment (direct evidence). RCTs provide the highest quality evidence for this comparison; or, if this is not available:

- evidence of treatment effectiveness from high-quality comparative studies evaluating the change in management for TB, linked with applicable and high-quality evidence of the accuracy of NAAT to diagnose MTB or NTM, compared with culture plus DST. This is called 'linked evidence'.

There was limited direct evidence available that met all the inclusion criteria that assessed the safety and effectiveness of NAAT in the diagnosis of MTB or NTB infections.

Review of literature

Literature sources and search strategies

The medical literature was searched to identify relevant studies and reviews for the period between 1990 and June 2014. Searches were conducted for the databases described in Table 5. Search terms are described in Table 6 to Table 10.

Due to the large volume of evidence for the diagnostic accuracy of NAAT compared with culture, only studies published after 2005 that provided 2x2 data suitable for meta-analysis for both AFB microscopy and NAAT compared with culture, were included in the final analysis. Studies on the only commercial NAAT product (Xpert) available in Australia were published in 2006 onwards. In-house NAAT, on the other hand, was available before 2005. However, as there have been significant changes in laboratory practice over the past 10 years (Boyle & Pai 2012; Moore, Guzman & Mikhail 2005; Nybo 2012; Public Health and Ambulatory Care 2012), it seemed reasonable to limit study eligibility to publications in the previous decade.

The diagnostic accuracy of in-house NAAT performed more than 10 years ago compared with culture was reported in two SRs. Pai et al. (2004) reported that the pooled sensitivity in pleural fluid specimens was 71% (95%CI 63, 76; k=26), and Pai et al. (2003) reported that the pooled sensitivity in CSF specimens was 76% (95%CI 57, 83; k=35). These values are much lower than that reported in this assessment for non-sputum specimens (90%; 95%CI 83, 94, k=44; see Figure 15). Thus, the inclusion of only those studies published after 2005 in the final analysis provided more-accurate data on the accuracy of NAAT as currently performed in the diagnostic laboratory.

An SR by Takwoingi et al. (2013) showed that there was a > 2-fold discrepancy in the relative diagnostic odds ratio between non-comparative studies (that compared either the index test or the comparator with the reference standard) and comparative studies (that compared both the index test and the comparator with the reference standard). Thus, these studies provide the highest quality evidence available to assess the accuracy of NAAT and AFB microscopy compared with culture to diagnose MTB infections.

Table 5 Electronic databases searched

Electronic database	Period covered
Cochrane Library – including, Cochrane Database of Systematic Reviews, Database of Abstracts of Reviews of Effects, the Cochrane Central Register of Controlled Trials (CENTRAL), the Health Technology Assessment Database, the NHS Economic Evaluation Database	1990 – 6/2014
Current Contents	1990 – 6/2014
Embase	1990 – 6/2014
PubMed	1990 – 6/2014
Web of Science – including Science Citation Index Expanded and Conference Proceedings Citation Index- Science	1990 – 6/2014
Cinahl	1990 – 6/2014
Econlit	1990 – 6/2014
Scopus	1990 – 6/2014

Table 6 Search terms used for NAAT for MTB (direct evidence, accuracy and change in management)

Element of clinical question	Search terms
Population	(tuberculosis OR MTB OR MTB/RIF OR "tubercle bacillus" OR Tuberculosis [MeSH] OR Mycobacterium OR "M. africanum" OR "M. bovis" OR "M. microti" OR "M. canettii" OR "M. caprae" OR "M. pinnipedii" OR "M. mungi")
Intervention	(Amplicor OR Amplified OR "Direct Test" OR "Direct Detection" OR TaqMan OR Xpert OR "nucleic acid amplification" OR NAAT OR "polymerase chain reaction" OR PCR OR "Nucleic Acid Amplification Techniques" [MeSH])
Comparator (if applicable)	N/A
Outcomes (if applicable)	N/A
Limits	1990 – June 2014; NOT (Other Animals NOT Humans)

MeSH = Medical Subject Heading, based on a Medline/PubMed platform; N/A = not applicable

Table 7 Search terms used for NAAT for NTM (direct evidence, accuracy and change in management)

Element of clinical question	Search terms
Population	("mycobacterium nontuberculous" OR "Mycobacterium Infections, Nontuberculous" [MeSH] OR "environmental mycobacteria" OR "mycobacteria other than tuberculosis" OR MOTT OR NTM OR NTMB OR "M. abscessus" OR "M avium" OR "M. chelonae" OR "M. flavescens" OR "M. fortuitum" OR "M. genavense" OR "M. gordonae" OR "M. haemophilum" OR "M. intracellulare" OR "M. kansasii" OR "M. malmoense" OR "M. marinum" OR "M. peregrinum" OR "M. scrofulaceum" OR "M. simiae" OR "M. smegmatis" OR "M. szulgai" OR "M. terrae" OR "M. ulcerans" OR "M. xenopi")
Intervention	(Amplicor OR Amplified OR "Direct Test" OR "Direct Detection" OR Xpert OR TaqMan OR "nucleic acid amplification" OR NAAT OR "polymerase chain reaction" OR PCR OR "Nucleic Acid Amplification Techniques" [MeSH])

Element of clinical question	Search terms
Comparator (if applicable)	N/A
Outcomes (if applicable)	N/A
Limits	1990 – June 2014; NOT (Other Animals NOT Humans)

MeSH = Medical Subject Heading, based on a Medline/PubMed platform; N/A = not applicable

Table 8 Search terms used for impact of early identification of drug resistance and alternative treatment

Element of clinical question	Search terms
Population	(tuberculosis OR MTB OR MTB/RIF OR "tubercle bacillus" OR Tuberculosis [MeSH] OR Mycobacterium OR "M. africanum" OR "M. bovis" OR "M. microti" OR "M. canettii" OR "M. caprae" OR "M. pinnipedii" OR "M. mungi") AND ("rpoB protein, Mycobacterium tuberculosis" [Supplementary Concept] OR rpoB OR resistant OR resistance OR "multidrug resistant" OR MDR OR "Drug resistance, Bacterial" [MeSH])
Intervention	N/A
Comparator (if applicable)	(Rifampin [MeSH] OR rifampicin OR benemycin OR rimactan OR tubacin OR rifadin OR rimactane OR isoniazid)
Outcomes (if applicable)	(infectious OR contagious OR contacts OR delay OR "excess morbidity" OR "excess mortality" OR public health OR outbreak)
Limits	1990 – June 2014; NOT (Other Animals NOT Humans)

MeSH = Medical Subject Heading, based on a Medline/PubMed platform; N/A = not applicable

Table 9 Search terms used for impact of early versus delayed treatment for TB

Element of clinical question	Search terms
Population	(tuberculosis OR MTB OR MTB/RIF OR "tubercle bacillus" OR Tuberculosis [MeSH] OR Mycobacterium OR "M. africanum" OR "M. bovis" OR "M. microti" OR "M. canettii" OR "M. caprae" OR "M. pinnipedii" OR "M. mungi")
Intervention and comparator	(early OR delayed OR delay OR immediate OR timely OR speed OR expedited) AND (antibiotics OR drug OR treatment OR isoniazid OR rifampicin OR rifampin OR ethambutol OR myambutol OR pyrazinamide)
Outcomes (if applicable)	N/A
Limits	1990 – June 2014; NOT (Other Animals NOT Humans)

MeSH = Medical Subject Heading, based on a Medline/PubMed platform; N/A = not applicable

Table 10 Search terms used for impact of inappropriate antibiotic use

Element of clinical question	Search terms
Population	(tuberculosis OR MTB OR MTB/RIF OR "tubercle bacillus" OR Tuberculosis [MeSH] OR Mycobacterium OR "M. africanum" OR "M. bovis" OR "M. microti" OR "M. canettii" OR "M. caprae" OR "M. pinnipedii" OR "M. mungi")
Intervention	(antibiotics OR drug OR treatment OR isoniazid OR rifampicin OR rifampin OR ethambutol OR myambutol OR pyrazinamide)
Outcomes (if applicable)	("adverse events" OR side-effects OR reaction* OR "Drug-Related Side Effects and Adverse Reactions"[MeSH])
Study type	"systematic review" OR "meta-analysis"
Limits	1990 – June 2014; NOT (Other Animals NOT Humans)

MeSH = Medical Subject Heading, based on a Medline/PubMed platform; N/A = not applicable

Selection criteria

In general, studies were excluded if they:

- did not address the research question;
- focused on latent TB (as NAAT for latent TB has already been assessed);
- did not provide information on the pre-specified target population (i.e. were focused on mycobacteria that cause Hansen's disease (leprosy);
- did not address one of the pre-specified outcomes and/or provided inadequate data on these outcomes;
- were in a language other than English and were of a lower level of evidence than the included studies; or
- did not have an appropriate study design.

If the same data were duplicated in multiple articles, only results from the most comprehensive or most recent article were included.

Specified *a priori* patient subgroups of particular interest in the analysis included patients with high and low pre-test probabilities of having TB and patients with HIV.

Search results

The PRISMA flowcharts are shown in Figure 6, Figure 7, Figure 8, Figure 9 and Figure 10. These outline the study selection process and number of papers considered at each stage of the SR (Liberati et al. 2009).

PRISMA flowcharts Source: Liberati et al. (2009)

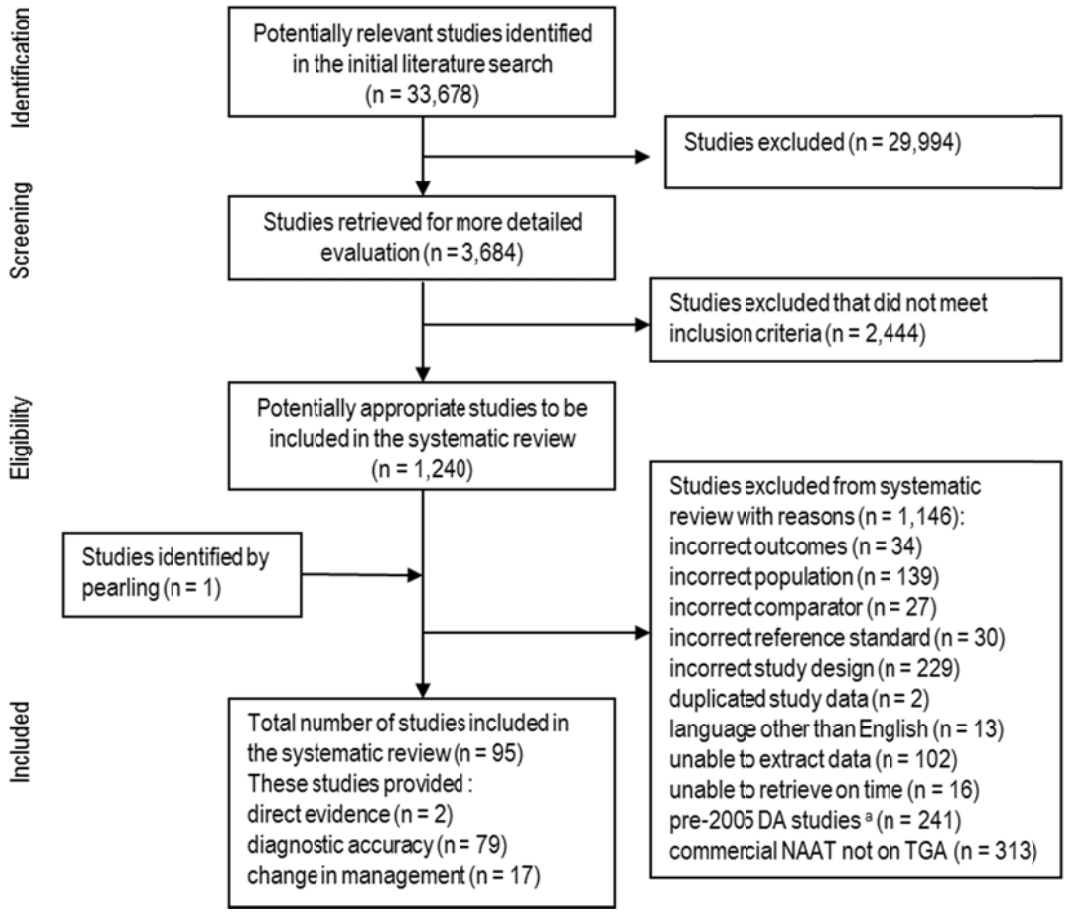

Figure 6 Summary of the process used to identify and select studies investigating the use of NAAT to diagnose MTB (direct evidence, accuracy and change in management)

[a] Pre-2005 DA studies were not further evaluated for inclusion (see 'Literature sources and search strategies')

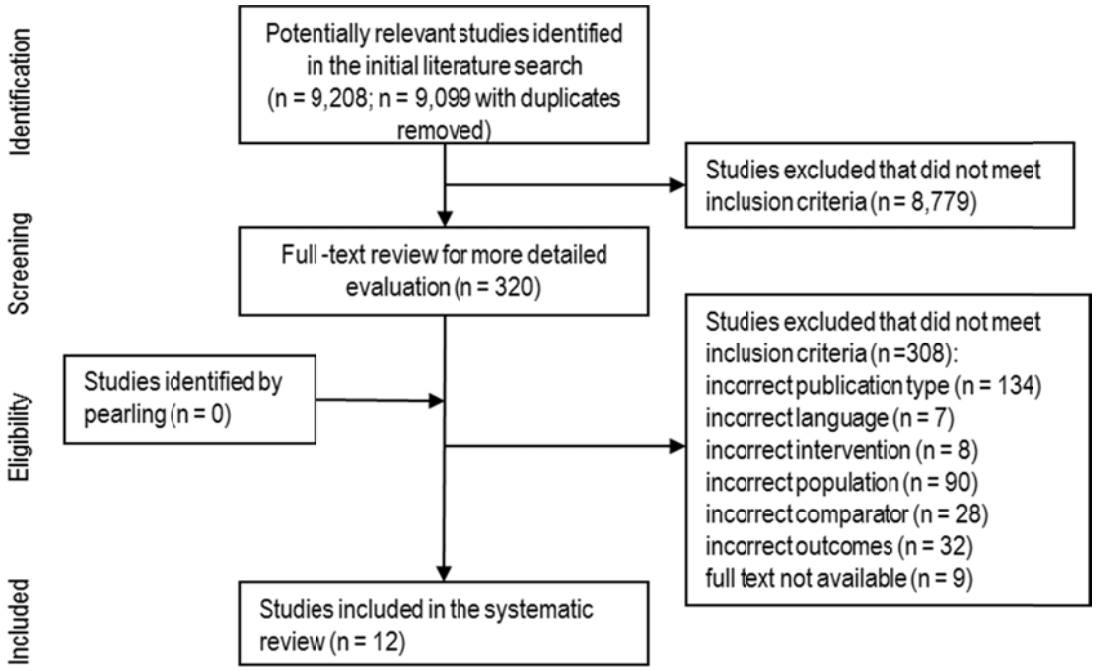

Figure 7 Summary of the process used to identify and select studies investigating the use of NAAT to diagnose NTM (direct evidence, accuracy and change in management)

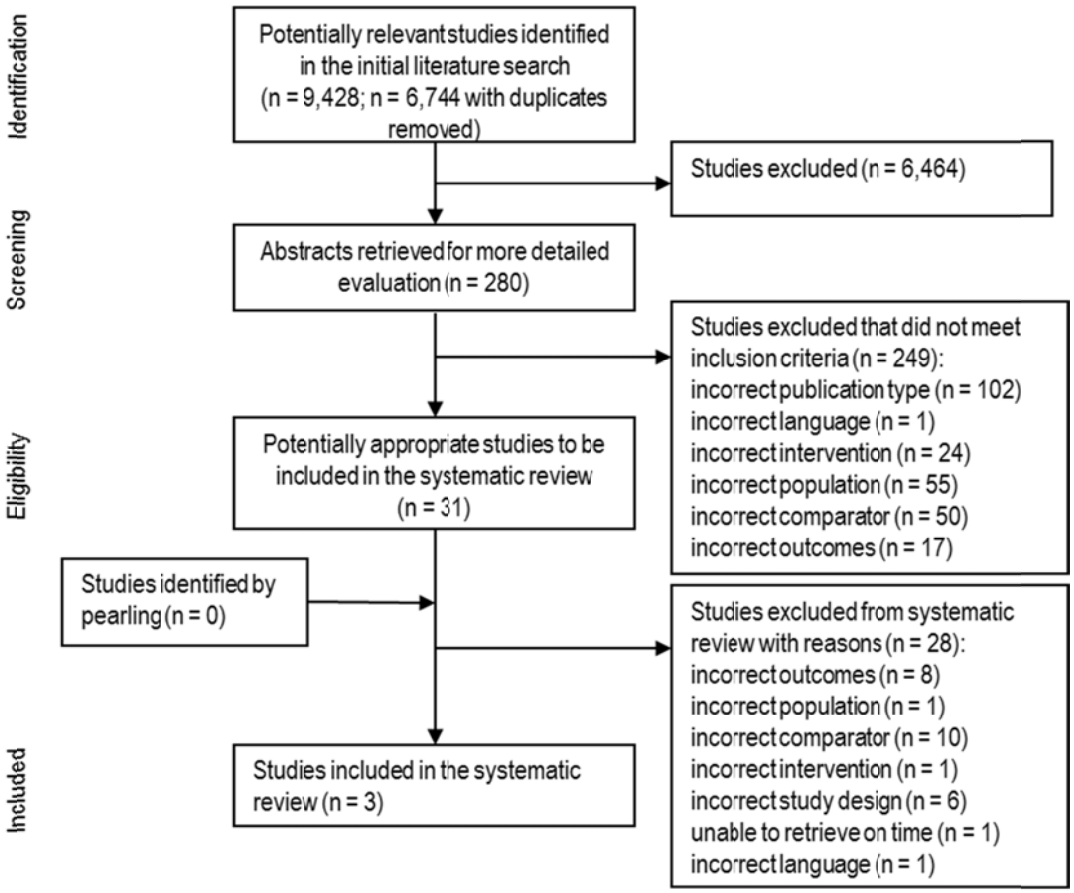

Figure 8 Summary of the process used to identify and select studies for the impact of early identification of drug resistance and alternative treatment

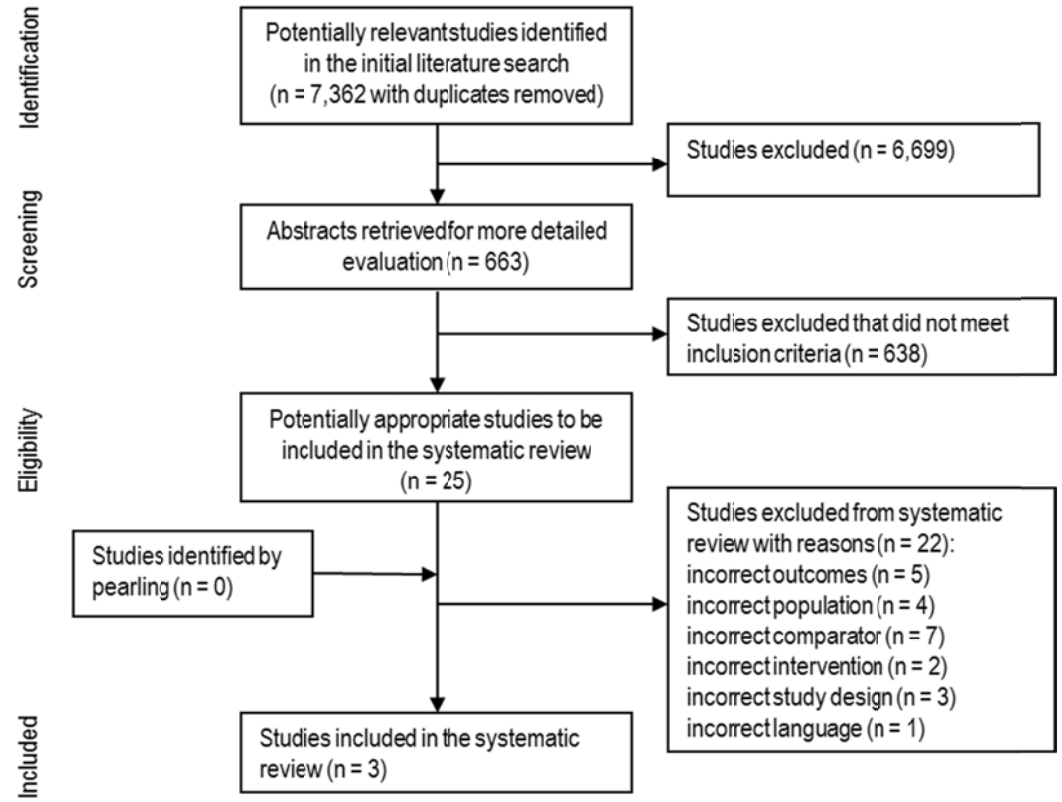

Figure 9 Summary of the process used to identify and select studies for the impact of early versus delayed treatment for TB

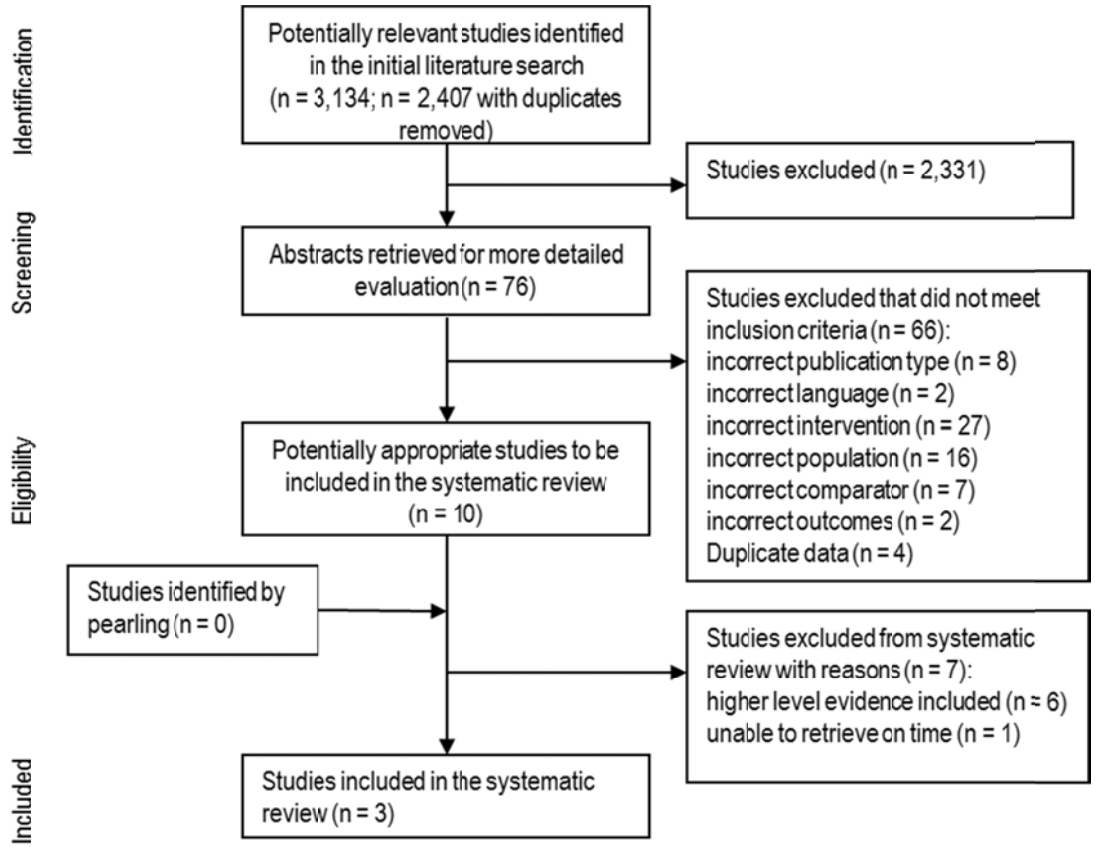

Figure 10 Summary of the process used to identify and select studies for the impact of inappropriate antibiotic use

Data extraction and analysis

A profile of key characteristics was developed for each included study (see Appendix F). Each study profile provides the level of evidence, design and quality of the study, authors, publication year, location, criteria for including/excluding patients, study population characteristics, type of intervention, comparator intervention and/or reference standard (where relevant), and outcomes assessed. Studies that could not be retrieved or that met the inclusion criteria but contained insufficient or inadequate data for inclusion are listed in Appendix G. Definitions of all technical terms and abbreviations are provided in the Glossary (page 30). Descriptive statistics were extracted or calculated for all safety and effectiveness outcomes in the individual studies.

Assessing diagnostic accuracy

To assess the diagnostic accuracy of NAAT, studies were only included if they provided data that could be extracted into a classic 2×2 table (Table 11), in which the results of the index diagnostic test or the comparator were cross-classified against the results of the reference standard (Armitage, Berry & Matthews 2002; Deeks 2001), and Bayes' Theorem was applied:

Table 11 Diagnostic accuracy data extraction for NAAT

		Reference standard (culture ± DST)		
		Disease +	*Disease –*	
Index test (NAAT)	*Test +*	true positive	false positive	Total test positive
Or comparator (AFB)	*Test –*	false negative	true negative	Total test negative
		Total with MTB or NTM	Total without MTB or NTM	

AFB = acid-fast bacilli; DST = drug susceptibility testing; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification testing; NTM = non-tuberculous mycobacteria

Primary measures

Test sensitivity was calculated as the proportion of people with MTB or NTM infections (as determined by the reference standard) who had a positive test result using AFB and/or NAAT:

Sensitivity (true positive rate) = number with true positive result / total with MTB or NTM infections

Test specificity was calculated as the proportion of people without infection (as determined by reference standard) who had a normal test result using AFB and/or NAAT:

Specificity (true negative rate) = number with true negative result / total without MTB or NTM infections

The 95%CI was calculated by exact binomial methods.

Positive and negative likelihood ratios (LR+ and LR–) were also reported. These ratios measure the probability of the test result in patients with MTB or NTM infections compared with those without.

LR+ = sensitivity / 1 – specificity

LR– = 1 – sensitivity / specificity

An LR of 1 means that the test does not provide any useful diagnostic information, whereas LR+ > 5 and LR– < 0.2 can suggest strong diagnostic ability (MSAC 2005).

Summary measures

Diagnostic test accuracy meta-analysis was undertaken to assess the accuracy of NAAT compared with AFB microscopy in the diagnosis of MTB or NTM infections, compared with culture, using Stata version 12 (Stata Corporation 2013). Only studies that provided raw (2×2) data were included. Summary receiver–operator characteristic (SROC) curves, forest plots and LR scattergrams were generated using the 'midas' command in Stata, which requires a minimum of four studies for analysis and calculates summary operating sensitivity

and specificity (with confidence and prediction contours in SROC space). Heterogeneity was calculated using the formula I^2 = 100% x (Q – df)/Q, where Q is Cochran's heterogeneity statistic and df is the degrees of freedom (Higgins et al. 2003). Summary estimates for sensitivity, specificity, LR+ and LR– were also calculated. Confidence intervals were computed assuming asymptotic normality after a log transformation for variance parameters and for LR+ and LR–.

Subgroup analyses were performed for results according to specimen type, incidence of TB in the study population and the presence of an HIV infection.

Where meta-analysis could not be performed, the median (range) sensitivity and specificity values were calculated.

Appraisal of the evidence

Appraisal of the evidence was conducted in three stages:

Stage 1: Appraisal of the applicability and quality of individual studies included in the review (strength of the evidence).

Stage 2: Appraisal of the precision, size of effect and clinical importance of the results for primary outcomes in individual studies—used to determine the safety and effectiveness of the intervention.

Stage 3: Integration of this evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

Stage 1: strength of the evidence

The evidence presented in the selected studies was assessed and classified using the dimensions of evidence defined by the National Health and Medical Research Council (NHMRC 2000).

These dimensions (Table 12) consider important aspects of the evidence supporting a particular intervention and include three main domains: strength of the evidence, size of the effect and relevance of the evidence. The first domain is derived directly from the literature identified as informing a particular intervention; the last two each require expert clinical input as part of its determination.

Table 12 Evidence dimensions

Type of evidence	Definition
Strength of the evidence:	
Level	The study design used, as an indicator of the degree to which bias has been eliminated by design [a]
Quality	The methods used by investigators to minimise bias within a study design
Statistical precision	The *p*-value or, alternatively, the precision of the estimate of the effect. It reflects the degree of certainty about the existence of a true effect
Size of effect	The distance of the study estimate from the 'null' value and the inclusion of only clinically important effects in the confidence interval
Relevance of evidence	The usefulness of the evidence in clinical practice, particularly the appropriateness of the outcome measures used

[a] See Table 13

The three sub-domains (level, quality and statistical precision) are collectively a measure of the strength of the evidence.

The 'level of evidence' reflects the effectiveness of a study design to answer a particular research question. Effectiveness is based on the probability that the design of the study has reduced or eliminated the impact of bias on the results. The NHMRC evidence hierarchy provides a ranking of various study designs ('levels of evidence') by the type of research question being addressed (Table 13).

Table 13 Designations of levels of evidence according to type of research question (including table notes)

Level	Intervention [a]	Diagnostic accuracy [b]
I [c]	A systematic review of level II studies	A systematic review of level II studies
II	A randomised controlled trial	A study of test accuracy with: an independent, blinded comparison with a valid reference standard [d], among consecutive persons with a defined clinical presentation [e]
III-1	A pseudo-randomised controlled trial (i.e. alternate allocation or some other method)	A study of test accuracy with: an independent, blinded comparison with a valid reference standard [d], among non-consecutive persons with a defined clinical presentation [e]
III-2	A comparative study with concurrent controls: ▪ non-randomised, experimental trial [f] ▪ cohort study ▪ case-control study ▪ interrupted time series with a control group	A comparison with reference standard that does not meet the criteria required for level II and III-1 evidence
III-3	A comparative study without concurrent controls: ▪ historical control study ▪ two or more single arm study [g] ▪ interrupted time series without a parallel control group	Diagnostic case-control study [e]
IV	Case series with either post-test or pre-test/post-test outcomes	Study of diagnostic yield (no reference standard) [h]

Source: Merlin, Weston & Tooher (2009)

Explanatory notes:

[a] Definitions of these study designs are provided on pages 7-8 in 'How to use the evidence: assessment and application of scientific evidence' (NHMRC 2000) and in the accompanying Glossary.

[b] These levels of evidence apply only to studies assessing the accuracy of diagnostic or screening tests. To assess the overall effectiveness of a diagnostic test there also needs to be a consideration of the impact of the test on patient management and health outcomes (MSAC 2005; Sackett & Haynes 2002). The evidence hierarchy given in the 'Intervention' column should be used when assessing the impact of a diagnostic test on health outcomes relative to an existing method of diagnosis/comparator test(s). The evidence hierarchy given in the 'Screening' column should be used when assessing the impact of a screening test on health outcomes relative to no screening or alternative screening methods.

[c] A systematic review will only be assigned a level of evidence as high as the studies it contains, excepting where those studies are of level II evidence. Systematic reviews of level II evidence provide more data than the individual studies and any meta-analyses will increase the precision of the overall results, reducing the likelihood that the results are affected by chance. Systematic reviews of lower level evidence present results of likely poor internal validity, and thus are rated on the likelihood that the results have been affected by bias rather than whether the systematic review itself is of good quality. Systematic review quality should be assessed separately. A systematic review should consist of at least two studies. In systematic reviews that include different study designs, the overall level of evidence should relate to each individual outcome/result, as different studies and study designs might contribute to each different outcome.

[d] The validity of the reference standard should be determined in the context of the disease under review. Criteria for determining the validity of the reference standard should be pre-specified. This can include the choice of the reference standard(s) and its timing in relation to the index test. The validity of the reference standard can be determined through quality appraisal of the study (Whiting et al. 2003).

[e] Well-designed population based case-control studies (e.g. screening studies where test accuracy is assessed on all cases, with a random sample of controls) do capture a population with a representative spectrum of disease and thus fulfil the requirements for a valid assembly of patients. However, in some cases the population assembled is not representative of the use of the test in practice. In diagnostic case-control studies a selected sample of patients already known to have the disease is compared with a separate group of normal/healthy people known to be free of the disease. In this situation patients with borderline or mild expressions of the disease, and conditions mimicking the disease are excluded, which can lead to exaggeration of both sensitivity and specificity. This is called spectrum bias or spectrum effect because the spectrum of study participants will not be representative of patients seen in practice (Mulherin & Miller 2002).

[f] This also includes controlled before-and-after (pre-test/post-test) studies, as well as adjusted indirect comparisons (i.e. use A vs B and B vs C, to determine A vs C with statistical adjustment for B).

[g] Comparing single arm studies i.e. case series from two studies. This would also include unadjusted indirect comparisons (i.e. use A vs B and B vs C, to determine A vs C but where there is no statistical adjustment for B).

[h] Studies of diagnostic yield provide the yield of diagnosed patients, as determined by an index test, without confirmation of the accuracy of this diagnosis by a reference standard. These may be the only alternative when there is no reliable reference standard.

Note A: Assessment of comparative harms/safety should occur according to the hierarchy presented for each of the research questions, with the proviso that this assessment occurs within the context of the topic being assessed. Some harms (and other outcomes) are rare and cannot feasibly be captured within RCTs, in which case lower levels of evidence may be the only type of evidence that is practically achievable; both physical and psychological harms may need to be addressed by different study designs; harms from diagnostic testing include the likelihood of false positive and false negative results; harms from screening include the likelihood of false alarm and false reassurance results.

Note B: When a level of evidence is attributed in the text of a document, it should also be framed according to its corresponding research question, e.g. level II intervention evidence; level IV diagnostic evidence; level III-2 prognostic evidence.

Note C: Each individual study that is attributed a 'level of evidence' should be rigorously appraised using validated or commonly used checklists or appraisal tools to ensure that factors other than study design have not affected the validity of the results.

Source: Hierarchies adapted and modified from: Bandolier editorial (1999); NHMRC (1999); Phillips et al. (2001).

Individual studies assessing diagnostic effectiveness were graded according to pre-specified quality and applicability criteria (MSAC 2005), as shown in Table 14.

Table 14 Grading system used to rank included studies

Validity criteria	Description	Grading system
Appropriate comparison	Did the study evaluate a direct comparison of the index test strategy versus the comparator test strategy?	C1 direct comparison CX other comparison
Applicable population	Did the study evaluate the index test in a population that is representative of the subject characteristics (age and sex) and clinical setting (disease prevalence, disease severity, referral filter and sequence of tests) for the clinical indication of interest?	P1 applicable P2 limited P3 different population
Quality of study	Was the study designed to avoid bias? High quality = no potential for bias based on pre-defined key quality criteria Medium quality = some potential for bias in areas other than those pre-specified as key criteria Poor quality = poor reference standard and/or potential for bias based on key pre-specified criteria	Q1 high quality Q2 medium quality Q3 poor reference standard poor quality or insufficient information

The appraisal of intervention studies pertaining to treatment safety and effectiveness was undertaken using the Downs and Black (1998) checklist, which was used for trials and cohort studies. Studies of diagnostic accuracy were assessed using the QUADAS-2 quality assessment tool (Whiting et al. 2011), whereas SRs included in the last step of the linked analysis were assessed with the PRISMA checklist (Liberati et al. 2009).

Stage 2: precision, size of effect and clinical importance

Statistical precision was determined using statistical principles. Small CIs and p-values give an indication as to the probability that the reported effect is real and not attributable to chance (NHMRC 2000). Studies need to be appropriately powered to ensure that a real difference between groups will be detected in the statistical analysis.

For intervention studies it was important to assess whether statistically significant differences between patients receiving intervention and comparator were also clinically important. The size of the effect needed to be determined, as well as whether the 95%CI included only clinically important effects.

The outcomes being measured in this report were assessed as to whether they were appropriate and clinically relevant (NHMRC 2000).

Stage 3: Assessment of the body of evidence

Appraisal of the body of evidence was conducted along the lines suggested by the NHMRC in their guidance on clinical practice guideline development (NHMRC 2009). The five components considered essential by the NHMRC when judging the body of evidence are the:

- evidence-base—which includes the number of studies sorted by their methodological quality and relevance to patients
- consistency of the study results—whether the better quality studies had results of a similar magnitude and in the same direction i.e. homogenous or heterogeneous findings
- potential clinical impact—appraisal of the precision, size and clinical importance or relevance of the primary outcomes used to determine the safety and effectiveness of the test
- generalisability of the evidence to the target population
- applicability of the evidence—integration of this evidence for conclusions about the net clinical benefit of the intervention in the context of Australian clinical practice.

A matrix for assessing the body of evidence for each research question, according to the components above, was used for this assessment (Table 15).

Table 15 Body of evidence matrix

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]	One or more level I studies with a low risk of bias or several level II studies with a low risk of bias	One or two level II studies with a low risk of bias, or an SR or several level III studies with a low risk of bias	One or two level III studies with a low risk of bias, or level I or II studies with a moderate risk of bias	Level IV studies, or level I to III studies/SRs with a high risk of bias
Consistency [b]	All studies consistent	Most studies consistent and inconsistency may be explained	Some inconsistency reflecting genuine uncertainty around clinical question	Evidence is inconsistent
Clinical impact	Very large	Substantial	Moderate	Slight or restricted
Generalisability	Population(s) studied in body of evidence are the same as target population	Population(s) studied in the body of evidence are similar to target population	Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population [c]	Population(s) studied in body of evidence differ from target population and it is hard to judge whether it is sensible to generalise to target population
Applicability	Directly applicable to Australian healthcare context	Applicable to Australian healthcare context with few caveats	Probably applicable to Australian healthcare context with some caveats	Not applicable to Australian healthcare context

SR = systematic review; several = more than two studies

[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).

[b] If there is only one study, rank this component as 'not applicable'.

[c] For example, results in adults that are clinically sensible to apply to children OR psychosocial outcomes for one cancer that may be applicable to patients with another cancer.

Source: Adapted from NHMRC (2009)

Expert advice: Health Expert Standing Panel (HESP)

HESP has been established as a panel of the MSAC and is a pool of experts collated from various medical fields who are nominated by their associated professional body or by applicants. HESP members are engaged to provide practical, professional advice to evaluators that directly relates to each application and the service being proposed for the MBS. HESP members are not members of either MSAC or its subcommittees. Their role is limited to providing input and guidance to the assessment groups to ensure that the pathway is clinically relevant and takes into account consumer interests. HESP members' advice is used to inform the deliberations that MSAC presents to the Federal Minister for Health.

Results of assessment and discussion

Is it safe?

Summary—What is the safety of NAAT versus current testing in diagnosing MTB?

No studies were identified assessing the safety of NAAT versus current testing in patients suspected of TB. To date, NAAT has been widely used without any safety concerns.

Studies were screened to assess the safety of NAAT according to criteria outlined *a priori* in Box 1.

Box 1 PICO criteria for studies assessing the safety of NAAT in patients suspected of TB where AFB microscopy is obtained

Population	Patients with clinical signs and symptoms of active TB whose specimen is suitable for AFB microscopy and culture, and who have had < 3 days of anti-TB treatment
Intervention	AFB microscopy and culture plus NAAT for the detection of MTB-complex DNA and genetic mutations on the *rpoB* gene associated with rifampicin resistance
Comparators	AFB microscopy and culture
Outcomes	AEs from testing procedures and subsequent treatments
Publication type	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	1990 – May 2014 or inception of the database, if later than 1990
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

Safety of NAAT

No studies were identified that reported on the safety of NAAT (plus AFB microscopy and/or culture) compared with current testing (AFB microscopy, tissue biopsy and/or culture). As NAAT is usually conducted on the same samples used for other testing, no AEs were expected.

To date, NAAT has been widely used without any safety concerns. However, more patients will receive a false-positive NAAT than a false-positive AFB result. Therefore, more patients will receive treatment for a disease they do not have and will possibly have an adverse reaction to the anti-TB drugs until clinical unresponsiveness is noted or culture results become available.

Is it effective?

Direct evidence of the effectiveness of NAAT in the diagnosis of MTB

Summary—Does NAAT improve health outcomes?

Both studies assessing the direct health impact of NAAT were conducted in a setting with a high TB prevalence, and so the applicability to the Australian healthcare system is questionable.

A high-quality RCT reported no difference in morbidity outcomes at 2 and 6 months follow-up when NAAT and AFB microscopy were compared. However, a strong trend indicating fewer deaths in the NAAT group compared with the AFB microscopy group was observed at 2 months, but this trend was no longer apparent at 6 months. A historical control study of medium quality found no difference in the mortality rate at 2 months follow-up when comparing NAAT with no NAAT. However, both studies were likely to be confounded by high levels of treatment initiation based on clinical evidence in the comparator groups.

The difference in treatment initiation between groups in the study by Theron et al. (2014) is unlikely to be reflected in treatment initiation rates in Australia because NAAT is suggested to be used as an adjunct to AFB testing. The incremental impact of NAAT over current testing practice in Australia, and the impact on patient morbidity and mortality, cannot be estimated from this study.

Studies were included to assess the effectiveness of NAAT according to the criteria outlined *a priori* in Box 2.

Box 2 PICO criteria for identification of studies relevant to an assessment of effectiveness of NAAT for patients where AFB microscopy is obtained

Population	Patients with clinical signs and symptoms of active TB whose specimen is suitable for AFB microscopy and culture, and who have had < 3 days of anti-TB treatment
Intervention	AFB microscopy and culture plus NAAT for the detection of MTB-complex DNA and genetic mutations on the *rpoB* gene associated with rifampicin resistance
Comparators	AFB microscopy and culture
Outcomes	Time to symptom resolution, quality of life, length of infectious period, number of contacts infected
Publication type	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	1990 – June 2014 or inception of the database, if later than 1990
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

Two studies were included that assessed the direct health impact of NAAT on suspected TB patients (Theron et al. 2014; Yoon et al. 2012). However, both studies were conducted in a high-prevalence African setting, and so applicability to the Australian healthcare system is questionable. In the absence of studies conducted in a more relevant setting, the study

profiles of these two studies are summarised in Table 95 (Appendix F), and an overall summary of the body of evidence is presented in Table 16.

Table 16 Body of evidence matrix for studies reporting direct evidence on the effectiveness of NAAT in the diagnosis of MTB

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]		One or two level II studies with a low risk of bias, or an SR or several level III studies with a low risk of bias		
Consistency	All studies consistent			
Clinical impact				Slight or restricted
Generalisability				Population(s) studied in body of evidence differ from target population and it is hard to judge whether it is sensible to generalise to target population
Applicability				Not applicable to Australian healthcare context

[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13)
Source: Adapted from NHMRC (2009)

One of the studies was a high-quality multicentre RCT (conducted in South Africa, Zimbabwe, Zambia and Tanzania) with 1,502 participants, who were either assigned to AFB microscopy plus culture (n=758) or Xpert plus culture (n=744). TB-related morbidity and mortality were reported in both groups (Theron et al. 2014). The lack of AFB microscopy in the Xpert plus culture arm of the trial further limits the applicability of the findings to the proposed use of Xpert in Australia.

The second study of 477 participants was a historical control study of medium quality with some risk of bias, and was conducted in Uganda (Yoon et al. 2012). This study included consecutive hospitalised Ugandan patients with suspected TB in two phases. In the baseline phase Xpert results were not reported to clinicians, whereas in the implementation phase the results were reported. Two-month mortality was reported and compared between groups.

Morbidity

Theron et al. (2014) reported comparative morbidity outcomes after NAAT compared with AFB microscopy. In this study AFB microscopy and NAAT (Xpert) were done at point-of-care to assist same-day clinical decision-making and to improve patient retention and clinical outcomes. Thus, both the NAAT results in the intervention group and the AFB microscopy results in the comparator group were available on the same day as specimen collection.

Additionally, only about half the patients who initiated treatment did so on the basis of a positive Xpert result. TB-related morbidity was graded using the TBscore (range 0–13) (Wejse et al. 2008) and the Karnofsky performance score (KPS). The KPS subjectively rates the patient's performance according to their ability to perform normal daily activities, ability to work, assistance needs, and disease-related symptoms on a scale from 0% to 100% (Rudolf et al. 2013). Morbidity was measured at baseline and at 2 and 6 months. The results are shown in Table 17.

Table 17 TB-related morbidity at recruitment, 2 months and 6 months, according to baseline culture status in patients given anti-TB treatment, per group

	TBscore NAAT (N=744)	TBscore AFB (N=758)	p-value	KPS NAAT (N=744)	KPS AFB (N=758)	p-value
Baseline						
Patients given treatment	Median (IQR) 5 (4–7)	Median (IQR) 5 (4–7)	0.12	Median (IQR) 70 (50–80)	Median (IQR) 70 (50–80)	0.62
- culture-positive group	n=168 5 (4–7)	n=153 5 (4–7)	0.56	n=168 70 (57.5–90)	n=153 70 (60–80)	0.89
- culture-negative or contaminated group	n=151 5 (4–7)	n=170 5 (4–6)	0.08	n=151 70 (50–80)	n=170 60 (50–80)	0.59
2 months						
Patients given treatment	Median (IQR) 2 (0–3)	Median (IQR) 1 (0–3)	0.39	Median (IQR) 90 (80–90)	Median (IQR) 90 (80–90)	0.91
- culture-positive [a]	2 (0.25–3)	2 (0–3)	0.85	90 (80–90)	80 (70–90)	0.23
- culture-negative [b]	1 (0–3)	1 (0–7)	0.37	90 (80–90)	80 (70–90)	0.23
Per-patient change in score since recruitment in patients given treatment	Median (IQR) 4 (2–5)	Median (IQR) 3 (2–4)	0.17	Median (IQR) 10 (10–30)	Median (IQR) 20 (10–30)	0.87
- culture-positive	3 (2–5)	3 (2–4)	0.20	10 (10–30)	10 (0–22.5)	0.59
- culture-negative or contaminated	4 (2.5–5)	3 (2–4)	0.28	20 (10–30)	20 (10–30)	0.96
Patients with a > 25% decrease (for TBscore) or increase (for KPS) in score from baseline	n/N (%) 168/197 (85%)	n/N (%) 150/183 (82%)	0.38	n/N (%) 93/197 (47%)	n/N (%) 83/183 (45%)	0.72
- culture-positive	89/108 (82%)	66/87 (76%)	0.26	46/108 (43%)	32/87 (37%)	0.41
- culture-negative or contaminated	79/88 (90%)	84/96 (88%)	0.63	47/88 (53%)	51/96 (53%)	0.97
6 months						
Patients given treatment	Median (IQR) 0 (0–3)	Median (IQR) 1 (0–3)	0.20	Median (IQR) 100 (90–100)	Median (IQR) 100 (90–100)	0.81
- culture-positive [c]	1 (0–3)	1 (0–3)	0.35	100 (90–100)	100 (90–100)	0.85
- culture-negative [d]	0 (0–3)	0 (0–2)	0.80	100 (90–100)	100 (90–100)	0.87

	TBscore NAAT (N=744)	TBscore AFB (N=758)	p-value	KPS NAAT (N=744)	KPS AFB (N=758)	p-value
Per-patient change in score since recruitment in patients given treatment	Median (IQR) 4 (2–5)	Median (IQR) 4 (3–5)	0.16	Median (IQR) 30 (10–40)	Median (IQR) 30 (10–40)	0.92
- culture-positive	4 (2.25–5)	4 (3–5)	0.35	30 (10–40)	20 (10–40)	0.44
- culture-negative or contaminated	4 (3–5.5)	4 (3–5)	0.38	40 (17.5–50)	30 (20–40)	0.53
Patients with a > 25% decrease (for TBscore) or increase (for KPS) in score from baseline	n/N (%) 148/168 (88%)	n/N (%) 146/167 (87%)	0.85	n/N (%) 82/168 (49%)	n/N (%) 76/167 (46%)	0.55
- culture-positive	85/97 (88%)	70/81 (86%)	0.81	42/97 (43%)	32/81 (39%)	0.61
- culture-negative or contaminated	62/71 (87%)	76/86 (88%)	0.84	40/71 (56%)	44/86 (51%)	0.52

AFB = acid-fast bacilli; KPS = Karnofsky performance score, 0–100% = with 0% being dead, < 40% = unable to care for self and requires equivalent of institutional or hospital care, 50–70% = unable to work but able to live at home and care for most personal needs, = 80–90% able to carry on normal activity and to work, 100% = being normal with no signs of disease; NAAT = nucleic acid amplification testing; TB = tuberculosis; TBscore = score 0–13 based on the 13 clinical indications, each contributing 1 point, normal values are scored as zero

[a] 87 (57%) of the AFB microscopy group vs 108 (64%) of 168 in the NAAT group were followed up within 2 weeks (p=0.170); of the patients who were not followed up within 2 weeks, 11 (17%) of 66 vs 6 (10%) of 60 had died (p=0.274), and 33 (50%) of 66 vs 36 (60%) of 60 were followed up > 2 weeks before/after the specified date (p=0.260).

[b] 96 (56%) of 170 of the AFB microscopy group vs 88 (58%) of 151 in the NAAT group were followed up within 2 weeks (p=0.74); of the patients who were not followed up within 2 weeks, 15 (20%) of 74 vs 8 (13%) of 63 had died (p=0.237), and 22 (30%) of 74 vs 21 (33%) of 63 were followed up > 2 weeks before/after the specified date (p=0.651).

[c] 81 (53%) of 153 of the AFB microscopy group vs 97 (58%) of 168 in the NAAT group were followed up within 2 weeks (p=0.39); of the patients who were not followed up within 2 weeks, 14 (19%) of 72 vs 14 (20%) of 71 had died (p=0.967), and 23 (32%) of 72 vs 23 (33%) of 71 were followed up > 2 weeks before/after the specified date (p=0.954).

[d] 86 (51%) of 170 of the AFB microscopy group vs 71 (47%) of 151 in the NAAT group were followed up within 2 weeks (p=0.52); of the patients who were not followed up within 2 weeks, 21 (25%) of 84 vs 14 (18%) of 80 had died (p=0.241), and 28 (33%) of 84 vs 28 (35%) of 80 were followed up > 2 weeks before/after the specified date (p=0.822).

Source: Theron et al. (2014)

TBscores at baseline and at 2 months and 6 months follow-up were similar in both groups (Table 17). When both tests were compared there were no differences reported in the median per-patient change in TBscore or KPS. The proportion of patients with a > 25% decrease in TBscore or KPS from recruitment to 2 and 6 months follow-up also did not differ.

Mortality

In the RCT by Theron et al. (2014) mortality was reported after 2 and 6 months follow-up. A strong trend was observed indicating fewer deaths in the NAAT group at 2 months, but this did not quite reach statistical significance (Table 18 At 6 months there was no difference in the mortality rate between the two groups. The historical control study (Yoon et al. 2012) only followed patients for a duration of 2 months after testing and found no difference between the two groups (Table 18).

Table 18 Mortality after NAAT versus no NAAT

Study	NAAT group	Comparator	Relative risk (95%CI), p-value
Deceased at 2 months			
Theron et al. (2014)	14/321 (4%)	26/324 (8%)	0.543 (0.29, 1.02), p=0.0538
Yoon et al. (2012)	35/181 (19%)	55/278 (20%)	0.977 (0.67, 1.43), p=0.906
Deceased at 6 months			
Theron et al. (2014)	28/321 (9%)	35/324 (11%)	0.807 (0.50, 1.3), p=0.3737

Comparator for Theron et al. (2014) was AFB microscopy
Comparator for Yoon et al. (2012) was a historical control group

Discussion

There was little difference in the observed mortality and morbidity rates when the diagnosis of TB included the use of NAAT compared with no NAAT for patients in these two studies. The RCT showed a trend towards improved mortality rate with the use of NAAT at 2 months but this trend was not observed at 6 months. Both these studies were conducted in countries with a high prevalence of TB. The authors from both studies postulated various reasons for this general lack of effect on morbidity and/or mortality despite improved TB diagnosis and treatment initiation in the NAAT groups compared with the comparator groups. Theron et al. (2014) suggested that the potential long-term epidemiological effect of NAAT was probably underestimated in their study because of high levels of treatment initiation in AFB-negative patients in the comparator group.

It should also be noted that while 112 NAAT-positive patients out of a total of 170 culture-positive (eventually treated) patients (66%) started treatment on the same day, the availability of same-day AFB microscopy results in the comparator group resulted in 67 AFB-positive patients out of 154 culture-positive (eventually treated) patients (44%) also starting treatment on the same day. This approximate 20% difference in treatment initiation between groups is unlikely to be reflected in treatment initiation rates in Australia because NAAT is suggested to be used as an adjunct to AFB testing. The incremental impact of NAAT over current testing practice in Australia, and the impact on patient morbidity and mortality, cannot be estimated from the study by Theron et al. (2014).

Yoon et al. (2012) suggested that the lack of effect on 2-month mortality in their study may be due to several factors, including insufficient powering to detect small differences in mortality rates between groups, a significantly higher proportion of patients in the baseline phase receiving empiric TB treatment compared with the implementation phase, and more patients presenting with increased disease severity in the implementation phase than in the baseline phase. Thus, the authors concluded that the higher rates of empiric TB treatment in

the baseline phase and sicker patients in the implementation phase may have attenuated the 2-month mortality in the implementation group.

Due to the limited evidence provided by these two studies, a linked evidence approach was taken to inform this assessment.

Linked evidence of effectiveness of NAAT in the diagnosis of MTB

Is it accurate?

Summary—What is the diagnostic accuracy of NAAT (with or without AFB microscopy) versus culture compared with AFB versus culture in the detection of MTB?

Diagnostic accuracy meta-analyses were conducted for multiple comparisons and the results are summarised below.

Culture as the reference standard

Even though culture is considered to be the 'gold standard' diagnostic test for TB, it is an imperfect reference standard because not all patients who receive a clinical diagnosis of TB based on other findings such as histopathology, clinical symptoms and responsiveness to anti-TB drugs will be culture-positive.

The pooled sensitivity and specificity of culture and NAAT using clinical diagnosis as a reference standard showed that:

- 24% of patients clinically diagnosed with TB had a false-negative culture result compared with 14% having a false-negative NAAT.
- Thus, a large proportion of NAAT 'false-positive' patients (i.e. NAAT-positive, culture-negative) would be clinically diagnosed as having TB.

Therefore, NAAT is likely to be more effective at confirming the presence of an MTB infection than the meta-analysis using culture as the reference standard would suggest.

AFB microscopy plus NAAT compared with culture

The pooled sensitivity and specificity for AFB microscopy plus NAAT compared with culture was 94% (95%CI 91, 98) and 88% (95%CI 82, 92), respectively, and did not differ significantly to those for sputum and non-sputum specimens when analysed separately:

- 6% of patients will have a false-negative result and 12% of patients will have false-positive results.

The summary LR+ and LR– values for the ability of AFB plus NAAT to correctly diagnose the presence or absence of TB in patients when compared with culture suggest that:

- In sputum specimens AFB plus NAAT correctly identified most patients as either culture-positive or culture-negative.
- In non-sputum specimens AFB plus NAAT correctly identified most patients who were culture-negative and showed strong diagnostic evidence for confirmation of culture-positive TB.

Summary—What is the diagnostic accuracy of NAAT (with or without AFB microscopy) versus culture compared with AFB versus culture in the detection of MTB?

NAAT versus culture

Compared with culture the pooled sensitivity and specificity of NAAT for all specimens were 89% (95%CI 85, 92) and 94% (95%CI 91, 96), respectively, and did not differ significantly when sputum and non-sputum specimens were analysed separately:

- Overall, 11% of patients had false-negative results and 6% false-positive results.

The SROC curve showed some threshold effect, suggesting that in-house NAAT was less specific than commercial NAAT when compared with culture, especially in countries with a high incidence of TB and when testing non-sputum specimens.

The summary LR+ and LR– values for the ability of NAAT to correctly diagnose the presence or absence of TB in patients when compared with culture suggest that:

- Both in-house NAATs and the commercial Xpert NAAT had diagnostic value in confirming or excluding culture-positive disease.
- Overall, patients with a positive NAAT result were likely to have culture-positive TB, whereas patients with a negative NAAT result were unlikely to be falsely negative.

In the context of interpreting NAAT results in conjunction with AFB findings:

- When specimens are AFB-positive, NAAT could confidently exclude the likelihood of culture-positive TB, but a positive NAAT result did not eliminate the possibility of being culture-negative. The explanation for this result is that culture is an imperfect reference standard. Culture in AFB-positive specimens likely resulted in misclassification of many of the 22% false-positive results seen for NAAT.
- In AFB-negative specimens a positive NAAT result was likely to correctly confirm the presence of MTB. However, interpretation of a negative NAAT result is dependent on the type of specimen tested:
    - In patients with AFB-negative sputum a negative NAAT indicated that the patient may not be culture-positive but it could not be ruled out.
    - In patients with AFB-negative non-sputum specimens a negative NAAT result provided no additional useful information. This is likely due to the paucibacillary nature of AFB-negative specimens.

There was no difference in the diagnostic accuracy of NAAT compared with culture between HIV-positive and HIV-negative patients.

NAAT was both highly sensitive (93%; 95%CI 85, 97) and highly specific (98%; 95%CI 96, 99) compared with culture-based DST in identifying rifampicin-resistant MTB.

Comparison of NAAT, AFB microscopy and AFB plus NAAT using culture as the reference standard

AFB plus NAAT had the highest false-positive rate, at 12%, with NAAT at 6% and AFB at 2%:

- A false-positive result means a patient will receive treatment for a short time (until clinical unresponsiveness is noted or culture results are available) for a disease they do not have.

AFB microscopy had the highest false-negative rate, at 38%; NAAT and AFB plus NAAT were much lower at 11% and 6%m, respectively:

Summary—What is the diagnostic accuracy of NAAT (with or without AFB microscopy) versus culture compared with AFB versus culture in the detection of MTB?

- The consequences of a false-negative result are much more severe, as the patient may remain untreated for a longer time period and could potentially spread the disease to more individuals.

Studies were included to assess the accuracy of NAAT according to criteria outlined in Box 3.

Box 3 PICO criteria for identification of studies relevant to an assessment of the accuracy of NAAT

Population	Patients with clinical signs and symptoms of active TB who have a specimen suitable for AFB microscopy and culture, and who have had < 3 days of anti-TB treatment
Intervention	NAAT with or without AFB microscopy for the detection of MTB-complex DNA and genetic mutations associated with anti-TB drug resistance
Comparator	AFB microscopy
Reference standard	Culture ± DST
Outcomes[a]	- Sensitivity - Specificity - Positive/negative predictive value - Level of agreement (concordance of data) - Diagnostic yield
Publication type	All study designs listed in the 'Diagnostic accuracy' column of Table 13
Search period	2005 – June 2014
Language	Non-English language articles were excluded unless they provided a higher level of evidence than the English language articles identified

[a] Due to the large volume of studies, included studies were limited to those that provided 2x2 data suitable for meta-analysis of sensitivity, specificity and likelihood ratios

Pre-specified subgroups for analysis included patients with a high pre-test probability of active TB (e.g. those from a country with high rates of TB) versus those with a low pre-test probability of TB. Although studies conducted in countries with a high incidence of TB were a good surrogate for patients with a high pre-test probability of having TB, there was no good surrogate for patients with a low pre-test probability of having TB. Many of the patients in those studies conducted in countries with a low incidence of TB were most likely recent immigrants from high-incidence countries. Those studies that used extrapulmonary specimens were a more appropriate surrogate for patients with a low pre-test probability of having TB, as the incidence of TB was lower in these patients.

A total of 79 studies provided data to assess the diagnostic accuracy of NAAT and AFB microscopy compared with culture in mixed pulmonary and/or extrapulmonary specimens from patients suspected of having an MTB infection. Culture methods included standard diagnostic laboratory procedures such as L-J or Ogawa solid media and/or liquid BACTEC media. Of these 79 studies, 20 (10 using an in-house NAAT and 10 using the commercial Xpert NAAT) provided data using mixed pulmonary and extrapulmonary specimens, 34 (21 in-house NAAT and 13 Xpert) using sputum specimens and 40 (29 in-house NAAT and 11

Xpert) using non-sputum specimens. Eight studies only provided data for the accuracy of NAAT compared with culture in patients with AFB-negative specimens. Eleven studies (1 in-house NAAT 10 Xpert) assessed the diagnostic accuracy of NAAT compared with culture to identify patients with drug-resistant MTB infections; 3 of the included studies only provided data for this outcome. The study profiles, patient characteristics and quality appraisal of these studies are listed in Table 96 (Appendix F) and the extracted 2x2 data are presented in Appendix C (Table 71 to Table 90). An overall summary of the body of evidence is presented in Table 19.

Table 19 Body of evidence matrix for studies reporting on the accuracy of AFB and NAAT compared with culture in diagnosing MTB infections

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]	One or more level I studies with a low risk of bias or several level II studies with a low risk of bias			
Consistency		Most studies consistent and inconsistency may be explained		
Clinical impact		Substantial		
Generalisability		Population(s) studied in the body of evidence are similar to target population		
Applicability		Applicable to Australian healthcare context with few caveats		

[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).
Source: Adapted from NHMRC (2009)

The proportion of patients diagnosed with culture-positive MTB varied greatly among studies. In the 68 studies that compared the diagnostic accuracy of AFB microscopy and NAAT with culture in patients suspected of having TB, the proportion of patients from whom MTB could be cultured ranged from 1% to 81%, with a mean of 30%. The mean proportion of patients with culture-positive MTB infections was greater in countries with a high incidence of TB (> 100 cases per 100,000 people; 33%) than in those with intermediate (100–10 cases per 100,000 people; 29%) or low incidence (< 10 cases per 100,000 people; 24%) rates. As expected, the mean proportion of culture-positive specimens was greater in patients with AFB-positive specimens (80%, range 27–100%) than in AFB-negative specimens (19%, range 1–72%). The proportion of culture-positive specimens identified for all subgroups are listed in Table 93 (Appendix D).

Comparison of NAAT and culture, using clinical diagnosis as a reference standard

In order to compare the sensitivity and specificity of culture and NAAT, using clinical diagnosis as a reference standard, meta-analysis was conducted using data from 10 of the

included studies that provided data using a clinical reference standard. The basis for a positive clinical diagnosis differed between studies but included some or all of the following: clinical findings, AFB microscopy, histology/cytology, chest radiographic findings, site-specific CT scan / MRI results, culture results and response to anti-TB drug therapy. The pooled sensitivity for culture versus clinical diagnosis was 76% (95%CI 54, 90) compared with 86% (95%CI 77, 92) for NAAT versus clinical diagnosis (Figure 11). This indicated that 24% of patients clinically diagnosed with TB will have a false-negative culture result compared with 14% having a false-negative NAAT (1 – sensitivity). This finding was consistent with the proportion of culture-positive cases reported in the *Tuberculosis notifications in Australia, 2010 Annual Report*[10]; 78% of all MTB cases were confirmed by culture.

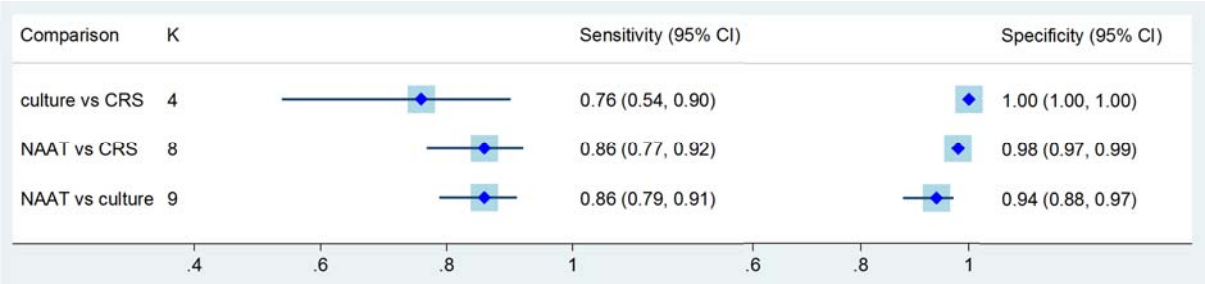

Figure 11 Forest plot showing the pooled sensitivity and specificity values for culture compared with NAAT, using a clinical reference standard, and for NAAT compared with culture in the same subset of studies

CRS = clinical reference standard; K = the number of studies; NAAT = nucleic acid amplification testing

Meta-analysis of studies assessing the diagnostic accuracy of AFB plus NAAT compared with culture

Forest plots showing the sensitivity and specificity for the 38 studies that compared the diagnostic accuracy of AFB microscopy plus NAAT with culture in patients suspected of having TB are shown in Figure 43 and Figure 44 (Appendix D). A summary of meta-analysis of subgroups based on NAAT methodology, specimen type and incidence of TB in the country the study was conducted in is presented in Figure 12. AFB microscopy combined with NAAT was very sensitive (overall: 94%; 95%CI 91, 98) but the specificity was lower (88%; 95%CI 82, 92). Thus, 6% of patients will have a false-negative result for both AFB microscopy and NAAT, and 12% of patients (8% with sputum specimens and 17% with non-sputum specimens) will be falsely-positive for either AFB microscopy or NAAT.

[10]Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

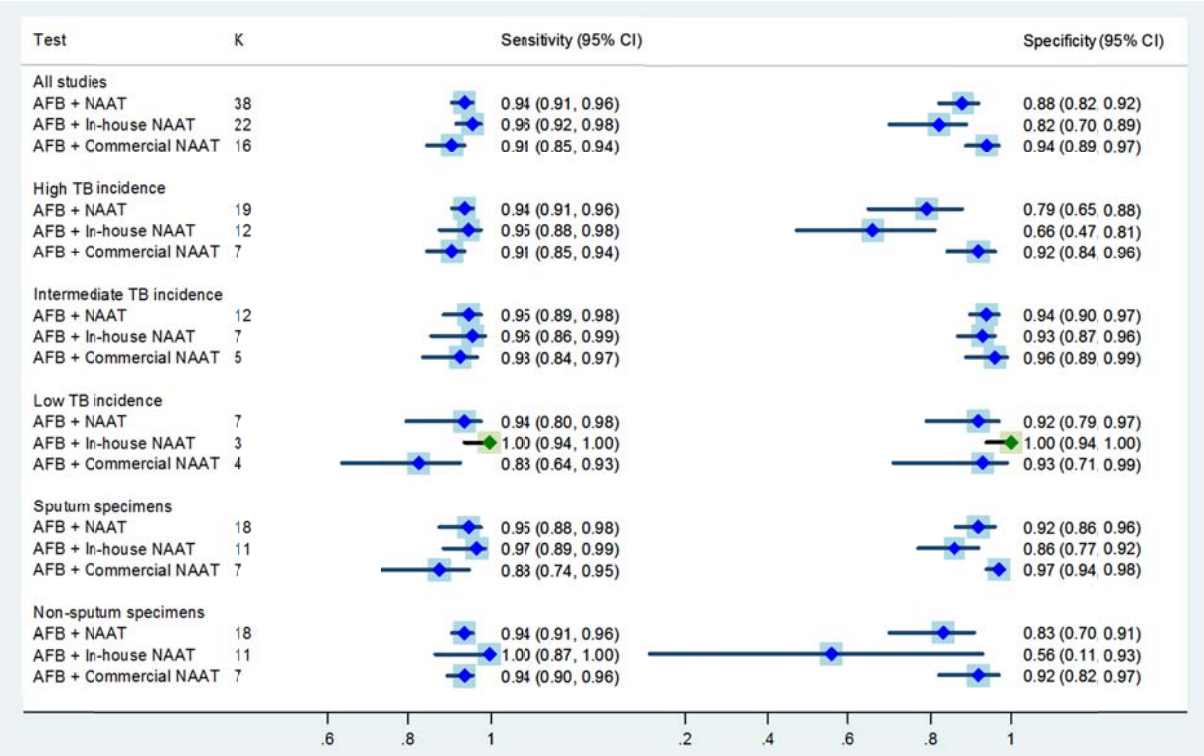

Figure 12 Forest plot showing the pooled sensitivity and specificity values for AFB plus NAAT compared with culture for studies grouped according to the NAAT comparator, specimen type and incidence of TB in the country in which the study was conducted

The sensitivity and specificity values shown in green represent median (range) values as meta-analysis could not be performed with that subgroup.

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; low incidence = ≤ 10 cases per 100,000 people

K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis

The LR scattergram in Figure 13 shows that the summary LR+ and LR– values for all studies investigating the ability of AFB microscopy plus NAAT to correctly identify patients with TB compared with culture were mostly within the green bands or the upper of the two left quadrants. This suggests that negative AFB and NAAT results correctly identified most patients who were culture-negative, and a positive result for either AFB or NAAT was more likely than not to indicate a culture-positive result. The reduced confidence in correctly diagnosing patients with culture-positive TB when AFB and NAAT were used together was due to the higher false-positive rate for the combined tests when compared with culture; 12% for AFB plus NAAT compared with 2% for AFB alone (Appendix E) and 6% for NAAT alone (Figure 15). As discussed above, culture is an imperfect reference standard and it is likely that many of the patients with apparent false-positive results actually have TB.

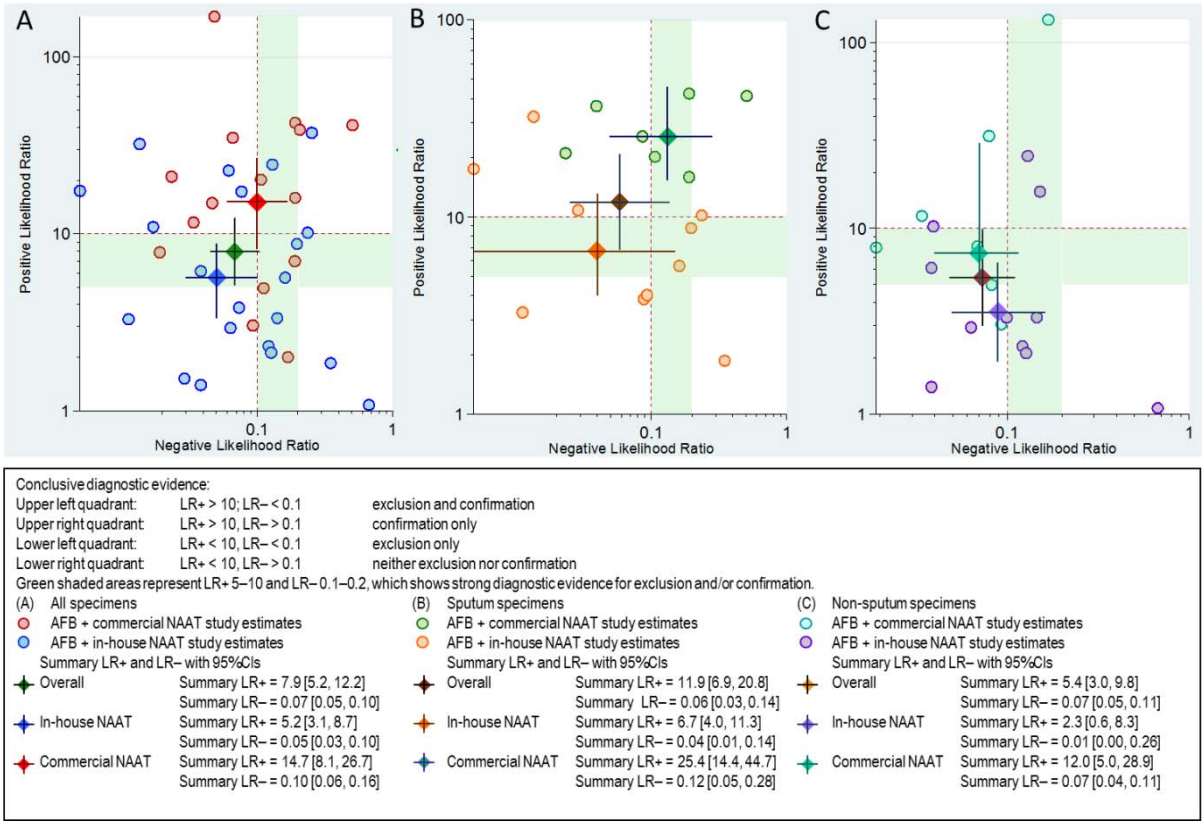

Figure 13 LR scattergram for diagnosis of MTB infection by AFB plus NAAT compared with culture in studies using either in-house NAAT or commercial Xpert NAAT

AFB microscopy plus NAAT was most effective at confirming and excluding the presence of culture-positive disease in sputum specimens but could only confidently exclude culture-positive disease in non-sputum specimens (Figure 13B and C). When studies using either an in-house NAAT or the commercial NAAT in combination with AFB were analysed separately, the summary LR+ and LR– estimates for the AFB plus commercial NAAT were more effective at confirming the presence of culture-positive disease than AFB plus in-house NAAT for all specimen types. Furthermore, in non-sputum specimens a positive AFB or in-house NAAT result did not provide any useful information, most likely due to the 14% false-positive rate in this population. A negative AFB and commercial NAAT result was only able to confidently exclude the presence of culture-positive disease in non-sputum specimens.

The SROC curve shows no threshold effect when AFB microscopy is combined with either in-house NAAT or commercial NAAT (Figure 14). The SROC curves also show that when AFB microscopy plus NAAT was conducted in countries with a high incidence of TB, the results were less sensitive in sputum specimens and less specific in non-sputum specimens than when conducted in countries with an intermediate or low incidence of TB. The area under the curve (AUC) for AFB microscopy plus NAAT (0.97; 95%CI 0.95, 0.98) indicated that AFB plus NAAT performs well in predicting culture positivity (AUC > 0.9).

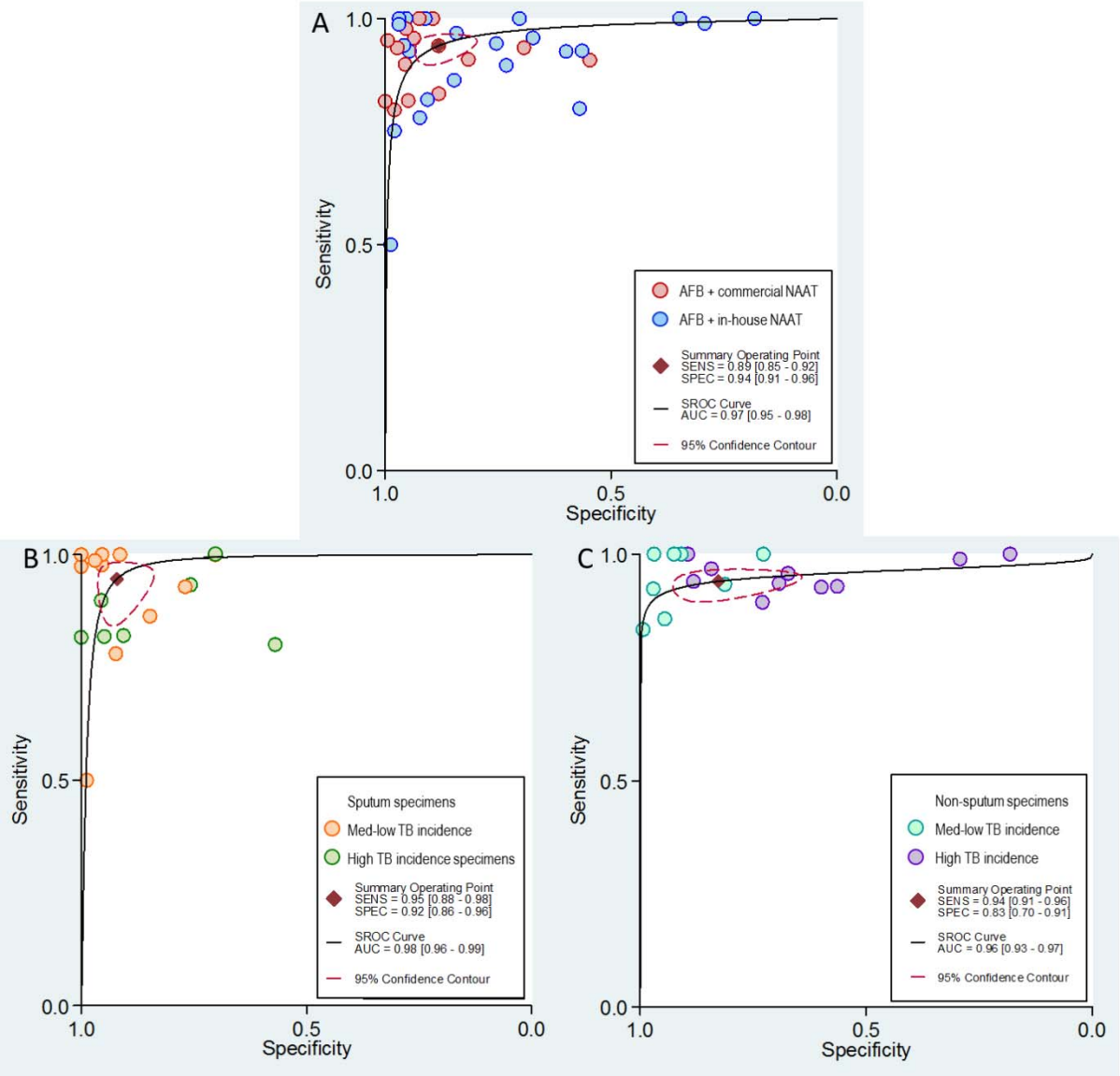

Figure 14 SROC curve for all studies investigating the sensitivity and specificity of AFB plus NAAT versus culture in the diagnosis of TB for all studies based on NAAT methodology (A), and for sputum (B) and non-sputum (C) specimens based on incidence of TB

AUC = area under curve; SROC = summary receiver–operator characteristic

Meta-analysis of studies assessing the diagnostic accuracy of NAAT compared with culture

Forest plots showing the sensitivity and specificity for the 68 studies that compared the diagnostic accuracy of NAAT with culture in patients suspected of having TB are shown in Figure 41 and Figure 42 (Appendix D). Although the sensitivity ranged from 6% to 100%, it was less variable than for AFB microscopy (Appendix E), with only 12/68 (18%) having a sensitivity below 70%. Meta-analysis showed that the overall pooled sensitivity for NAAT compared with culture was 89% (95%CI 85, 92). There were no significant differences in the pooled sensitivities for in-house compared with the commercial NAAT (Xpert) for any subgroup investigated; however, there was a slight trend suggesting that in-house NAATs were more sensitive than the commercial NAAT for most comparisons (Figure 15).

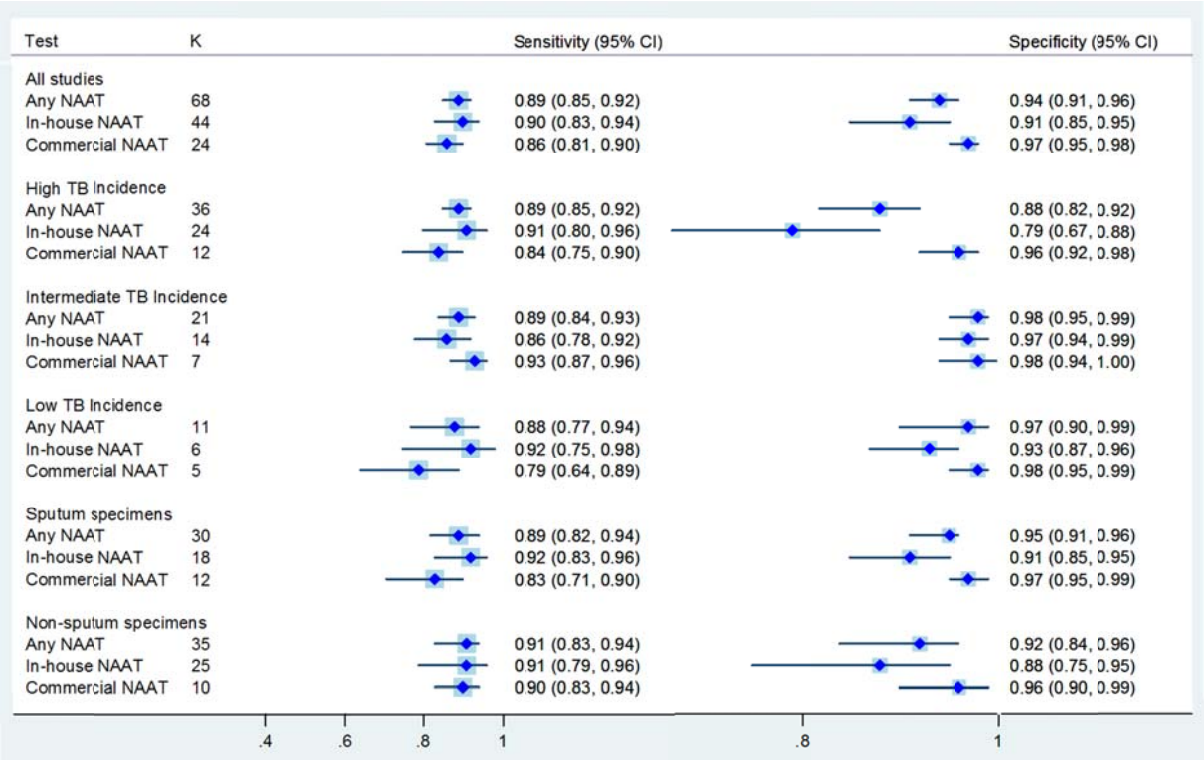

Figure 15 Forest plot showing the pooled sensitivity and specificity values for NAAT compared with culture for studies grouped according to the NAAT comparator, specimen type and incidence of TB in the country in which the study was conducted

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; low incidence = ≤ 10 cases per 100,000 people
K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis

There was greater variability in the specificity among studies, especially those that were conducted in countries with a high incidence of TB and that used in-house NAAT methodologies (Figure 42 in Appendix D). Meta-analysis of this subgroup showed that the pooled specificity was 79% (95%CI 67, 88), which was significantly lower than for the Xpert NAAT in these countries (96%; 95%CI 92, 96). In fact, the overall pooled specificity for all studies using in-house NAATs (91%; 95%CI 85, 95) was significantly lower than for those using the Xpert NAAT (97%; 95%CI 95, 98). Similar differences in the pooled estimates were seen for both the sputum and non-sputum subgroups, but the difference between in-house and commercial NAATs did not reach statistical significance in the non-sputum subgroup due to the wide CIs (Figure 15).

For specific non-sputum specimen types, the pooled specificity ranged from 90% to 97%, except for body fluids (such as synovial fluid and endometrial fluid), which had a pooled sensitivity of 69% (Figure 40 in Appendix D). It should be noted that although the pooled sensitivity of NAAT compared with culture was 97% for CSF specimens (compared with only 11% for AFB microscopy), the wide 95%CI (21, 100) indicated uncertainty in this estimate. As

expected, the pooled specificity among different specimen types was much more varied, ranging from 71% for body fluids to 97% for both bronchial specimens and urine specimens.

Overall, there was no observed publication bias based on the effective sample size between studies (p=0.23; Figure 16). However, when the studies were separated according to the use of an in-house or commercial NAAT index test, the slope became significant (p=0.05 and 0.02, respectively). There was no observed publication bias for the most variable subgroup as the slope of the regression line for in-house NAAT studies conducted in high TB incidence countries was non-significant (p=0.30).

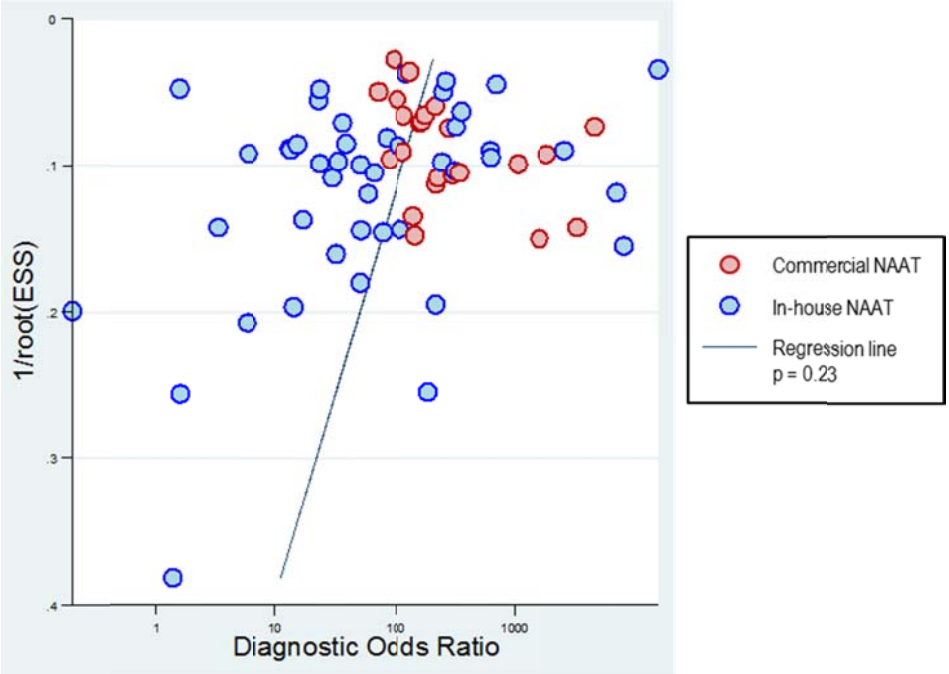

Figure 16 Deek's Funnel plot asymmetry test to assess publication bias for the diagnostic accuracy of NAAT compared with culture

Publication bias is assessed visually by using the inverse of the square root of the effective sample size (ESS) versus the log diagnostic odds ratio, which should have a symmetrical funnel shape when publication bias is absent (Light & Pillemer 1984). A regression slope coefficient, weighting by ESS, with $p<0.05$ indicates significant asymmetry (Deeks, Macaskill & Irwig 2005).

The LR scattergram in Figure 17 shows that the summary LR+ and LR– values for all studies investigating the ability of NAAT to correctly identify patients with and without TB, compared with culture, were mostly within the upper right quadrant of the graph (Figure 17A). Thus, a positive NAAT result was likely to correctly confirm the presence of MTB (as diagnosed by culture). As the summary estimates for exclusion were within the green shaded area close to the upper left quadrant, NAAT provided strong diagnostic evidence suggesting that patients who tested negative were more likely not to have culture-positive TB than to be falsely negative. Similar results were seen when studies that reported data for either sputum or non-sputum specimens were analysed separately (Figure 17B and C).

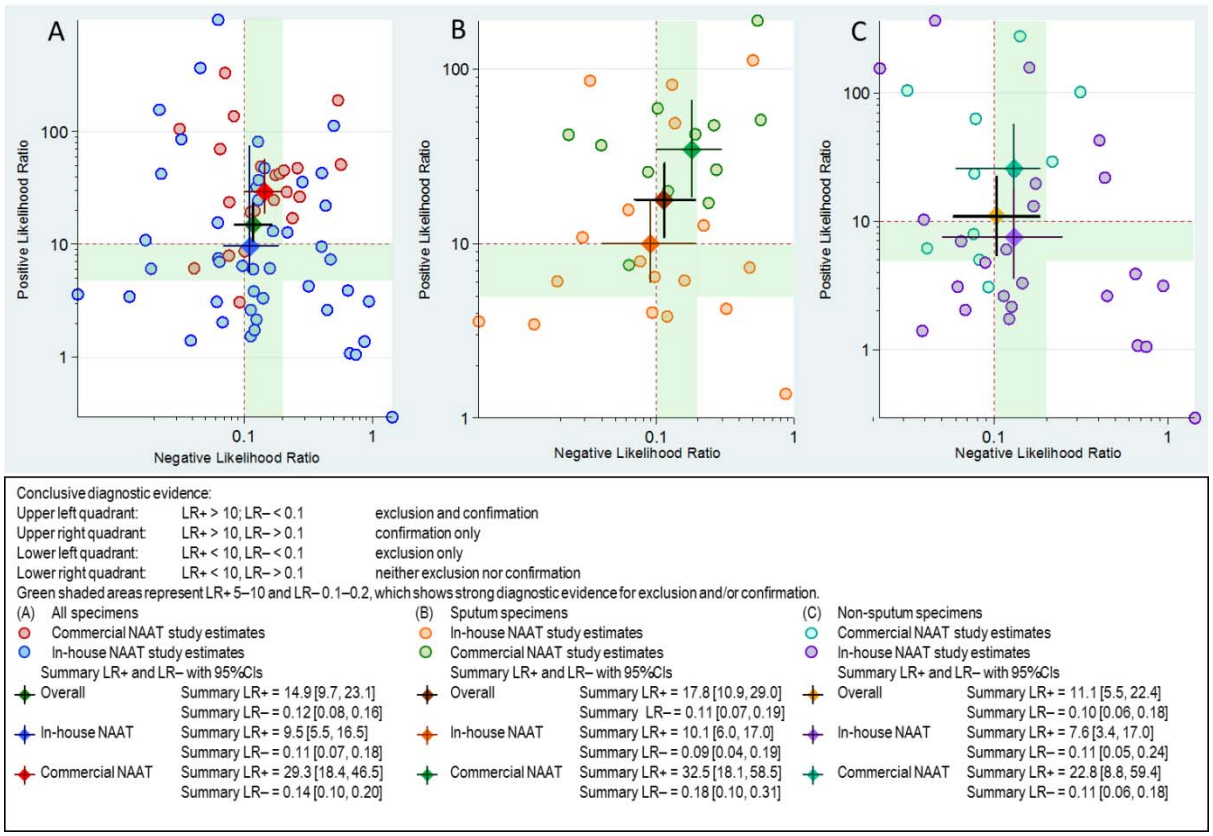

Figure 17 LR scattergram for diagnosis of MTB infection by NAAT compared with culture in studies using either in-house NAAT or commercial Xpert NAAT

LR = likelihood ratio; NAAT = nucleic acid amplification testing

The summary LR estimate for in-house NAATs was close to the border between the upper and lower quadrants or just below it, whereas the summary value for commercial NAAT was clearly in the upper half of the graph. This indicated that a positive NAAT result using commercial NAAT could predict culture-positivity with greater confidence than using an in-house NAAT for all specimen types. The summary estimates were also mostly within the green band in the upper right quadrant of the graph, indicating that although a negative result was likely to indicate a negative culture result, it could not rule out culture-positivity. For sputum specimens the summary estimate for in-house NAAT was just within the upper left quadrant, indicating more confidence in the specimen also being culture-negative.

The SROC curve, which depicts the relative trade-off between true-positive and false-positive results, indicated that NAAT performs well in predicting culture positivity, with an AUC of 0.97 (95%CI 0.95, 0.98) for all specimen types, 0.96 (95%CI 0.94, 0.97) for sputum specimens and 0.89 (95%CI 0.86, 0.91) for non-sputum specimens. The SROC curve showed some threshold effect, suggesting that in-house NAAT was less specific than the commercial Xpert NAAT when compared with culture, especially in countries with a high incidence of TB and when testing non-sputum specimens (Figure 18).

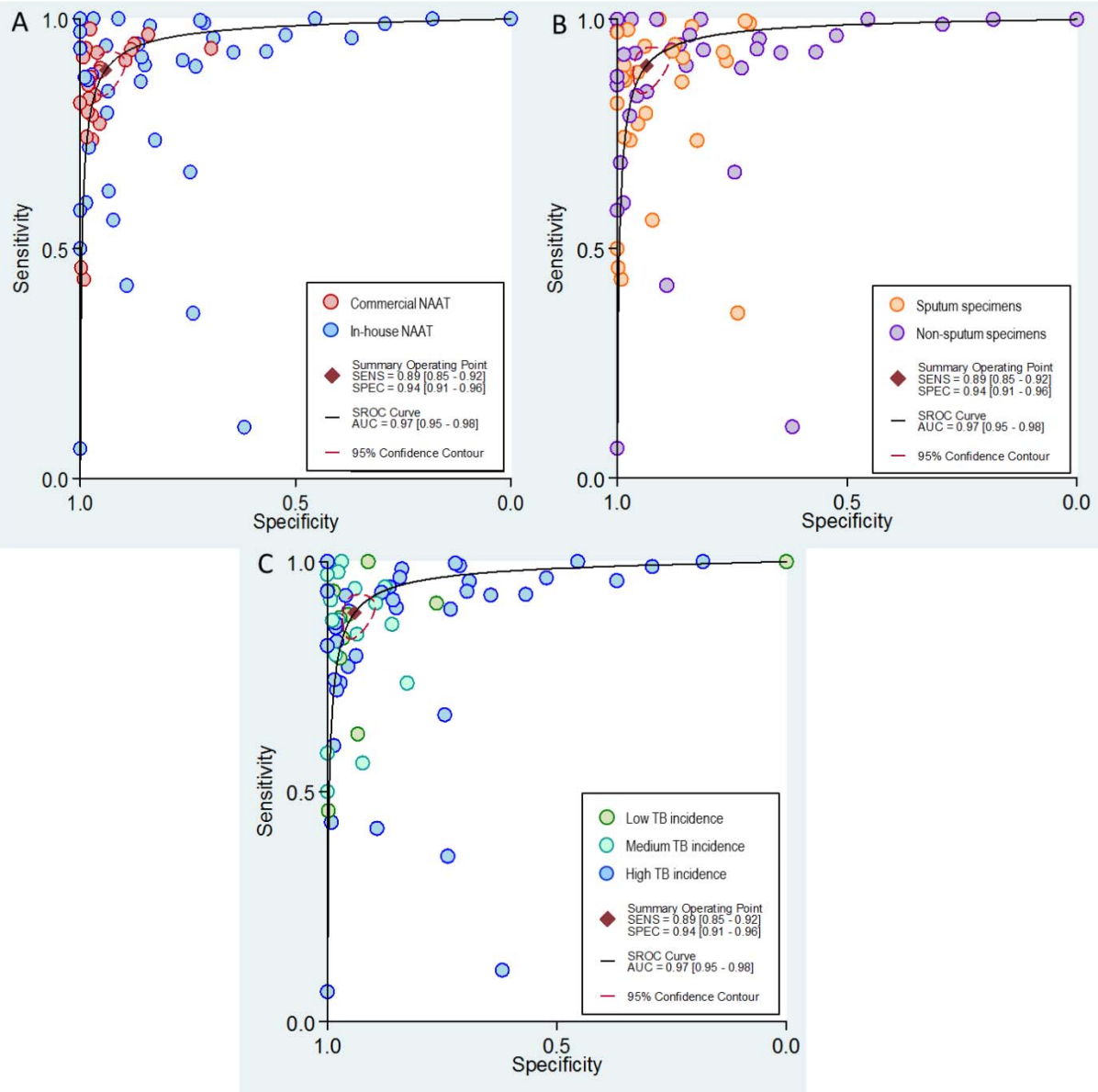

Figure 18 SROC curve for all studies investigating the sensitivity and specificity of NAAT versus culture in the diagnosis of TB for studies based on NAAT methodology (A), specimen type (B) and incidence of TB (C)

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; low incidence = ≤ 10 cases per 100,000 people

AUC = area under curve; NAAT = nucleic acid amplification testing; SROC = summary receiver–operator characteristic; TB = tuberculosis

In summary, the sensitivity and specificity for in-house NAATs and the commercial Xpert NAAT, when compared with culture, did not differ significantly. Nevertheless, the Xpert NAAT showed a trend suggesting that it may be less sensitive than in-house NAATs, especially when testing sputum specimens (83% versus 92%; Figure 15). The summary LR values indicate that both in-house NAATs and the commercial Xpert NAAT have diagnostic value in confirming or excluding culture-positive disease. Patients with a positive commercial NAAT result were more likely to be culture-positive than those with a positive in-house NAAT result for all specimen types. Patients with a negative NAAT result in sputum

specimens were more likely to be culture-negative than those with a negative Xpert NAAT result.

Meta-analysis of studies assessing the diagnostic accuracy of NAAT compared with culture in either AFB-positive or AFB-negative specimens

Forest plots showing the sensitivity and specificity from individual studies that compared NAAT with culture in either AFB-positive specimens or AFB-negative specimens from patients suspected of having TB are shown in Figure 45 and Figure 46 (Appendix D). Figure 19 shows the pooled sensitivity and specificity values for NAAT compared with culture for AFB-positive and AFB-negative specimens.

Among the 28 studies that reported data for AFB-positive specimens, the sensitivity was at least 94% in all but 5 studies (pooled value 99%; 95%CI 96, 100). However, the specificity was much more variable, ranging from 0% to 100% between studies (pooled value 78%; 95%CI 53, 92). Conversely, in the 39 studies that reported data for AFB-negative specimens, the sensitivity was highly variable between studies, with a pooled value of 80% (95%CI 69, 87). The specificity was at least 82% in the studies that were conducted in countries with a low or medium incidence of TB, but was highly variable (range 18–100%) in studies conducted in countries with a high incidence of TB, especially those using in-house NAAT. These observations are reflected in the 95%CIs of the pooled sensitivity and specificity values from subgroup analyses shown in Figure 19A and B.

The LR scattergram shows that the summary LR+ and LR– values for NAAT compared with culture in AFB-positive specimens were within the lower left quadrant, indicating that a negative NAAT result can confidently exclude the likelihood of an MTB infection (as determined by culture) in patients who had an AFB-positive sample (Figure 20). Unexpectedly, a positive NAAT result does not eliminate the possibility of AFB-positive patients not having a detectable MTB infection (i.e. being culture-negative). This can be explained because culture is an imperfect reference standard, which likely resulted in misclassification of many of the 22% false-positive results (1 – specificity) seen for NAAT when compared with culture in AFB-positive specimens (Figure 19). Therefore, NAAT is likely to be more effective at confirming the presence of an MTB infection in these patients than the LR scattergram suggests.

In AFB-negative specimens the overall summary LR+ and LR– values for NAAT compared with culture were in the upper right quadrant of the scattergram or within the green shaded bands, indicating that a positive NAAT result is likely to correctly confirm the presence of MTB. However, interpretation of a negative NAAT result is dependent on the type of

specimen tested. In patients with AFB-negative sputum a negative NAAT indicated that the patient may not be culture-positive but it could not be ruled out (summary values are within the green shaded area; Figure 21). In patients with AFB-negative non-sputum specimens, a negative NAAT result provided no additional useful information. This is likely due to the paucibacillary nature of AFB-negative specimens. It should be noted that if few bacilli are present in the specimen, the possibility of a false-negative result would increase for all three tests.

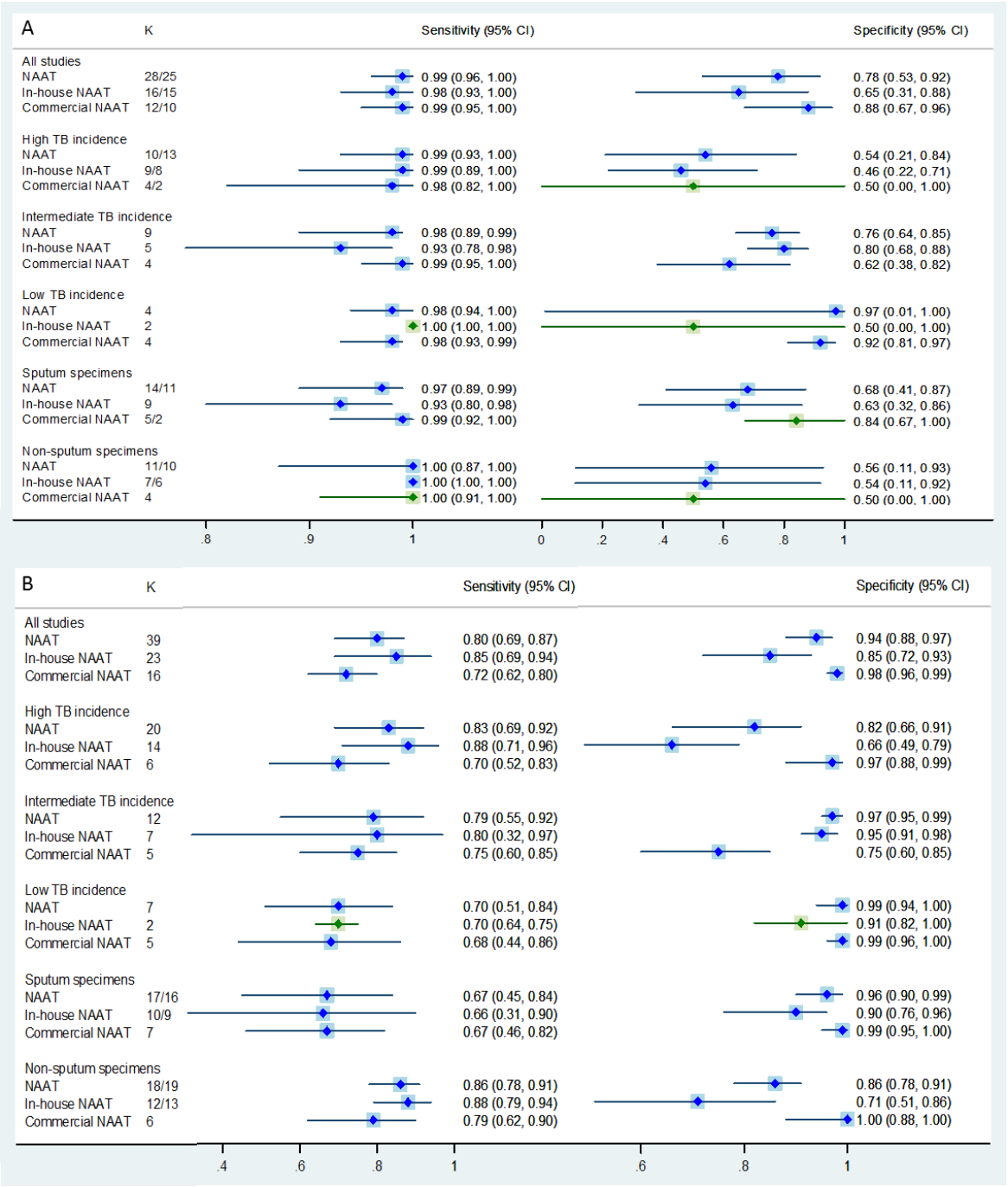

Figure 19 Forest plot showing the pooled sensitivity and specificity values for NAAT compared with culture for AFB-positive (A) and AFB-negative (B) specimens grouped according to NAAT methodology, specimen type and incidence of TB in the country in which the study was conducted

Green plots represent median (range) in groups for which meta-analysis could not be conducted.
Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; low incidence = ≤ 10 cases per 100,000 people
K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis

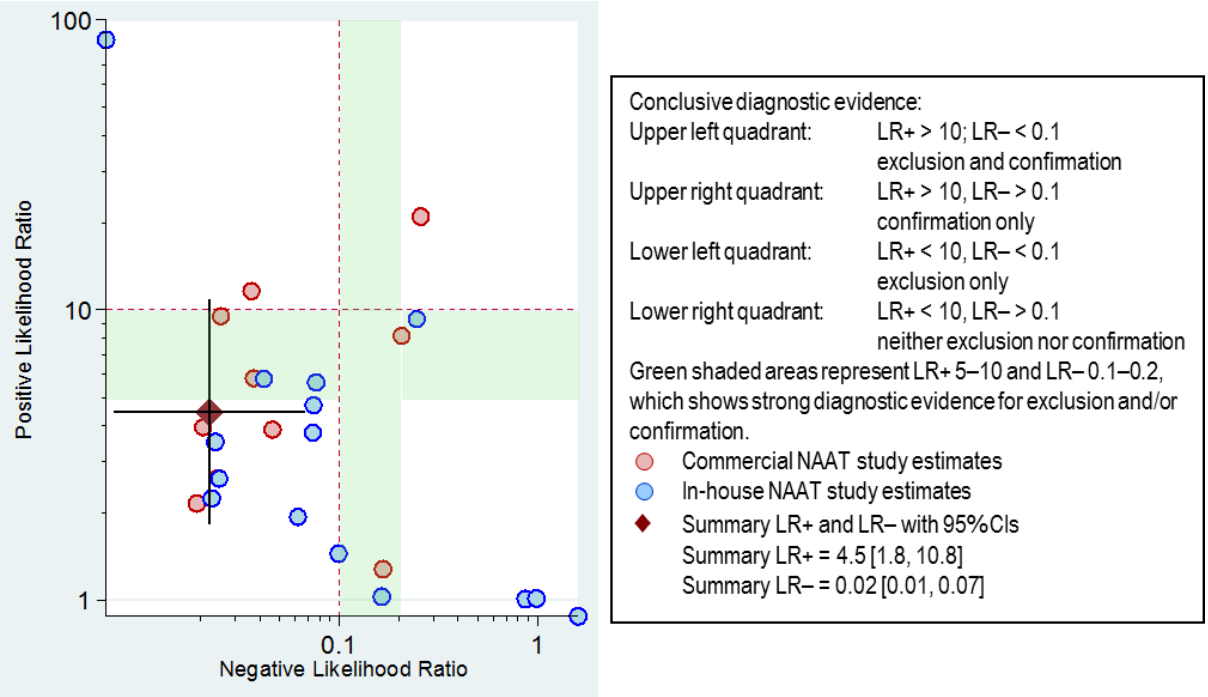

Figure 20 LR scattergram for diagnosis of MTB infection by NAAT compared with culture for AFB-positive specimens according to NAAT methodology

LR = likelihood ratio; NAAT = nucleic acid amplification testing

The SROC curve for studies investigating NAAT compared with culture in AFB-positive specimens showed no threshold effects based on commercial or in-house NAAT (Figure 22) or specimen type (not shown). However, for studies investigating NAAT compared with culture in AFB-negative specimens, a threshold effect was seen (Figure 23). In-house NAAT tended to be more sensitive and less specific than commercial NAAT when compared with culture. Similarly, NAAT compared with culture tended to be less sensitive and more specific when testing sputum specimens than for non-sputum specimens. The AUC for NAAT versus culture in AFB-positive (0.98; 95%CI 0.96, 0.99) and AFB-negative (0.93; 95%CI 0.91, 0.95) specimens indicated that the NAATs perform well in predicting culture positivity (AUC > 0.9) for both types of specimen.

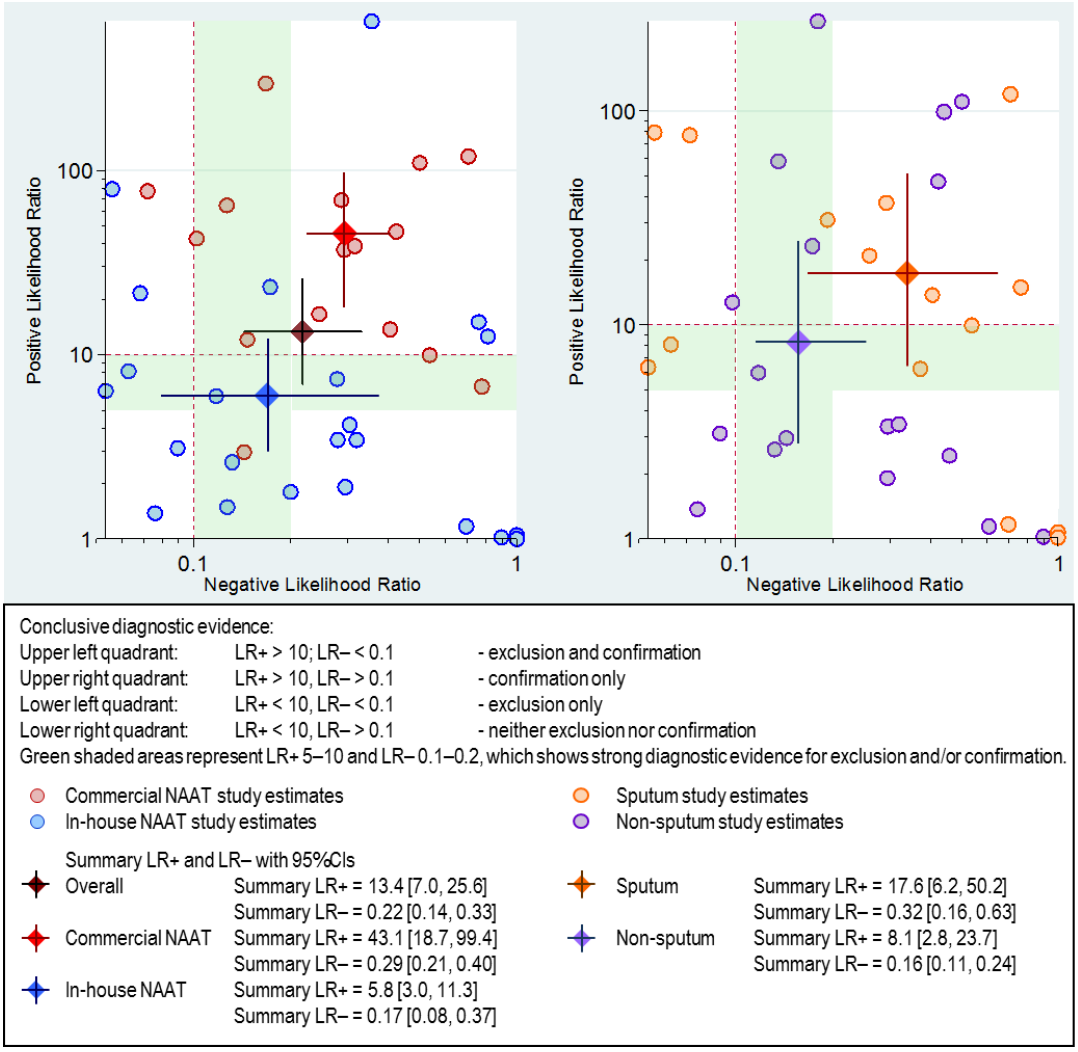

Figure 21 LR scattergram for diagnosis of MTB infection by NAAT compared with culture for AFB-negative specimens according to NAAT methodology

LR = likelihood ratio; NAAT = nucleic acid amplification testing

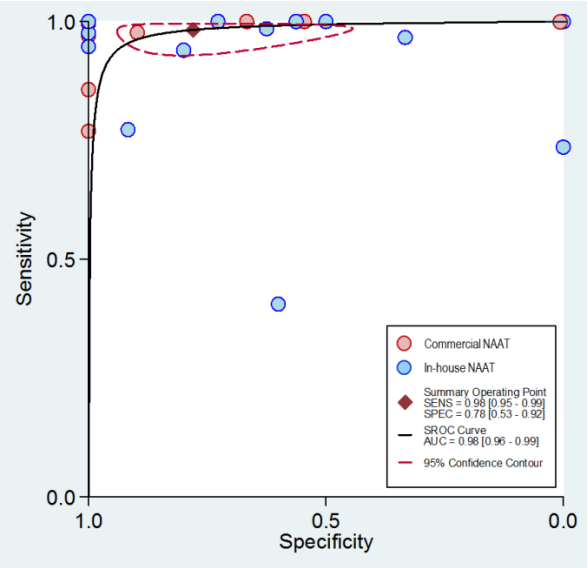

Figure 22 SROC curve for all studies investigating the sensitivity and specificity of NAAT versus culture in the diagnosis of TB for AFB-positive specimens based on NAAT methodology

AUC = area under curve; SROC = summary receiver–operator characteristic

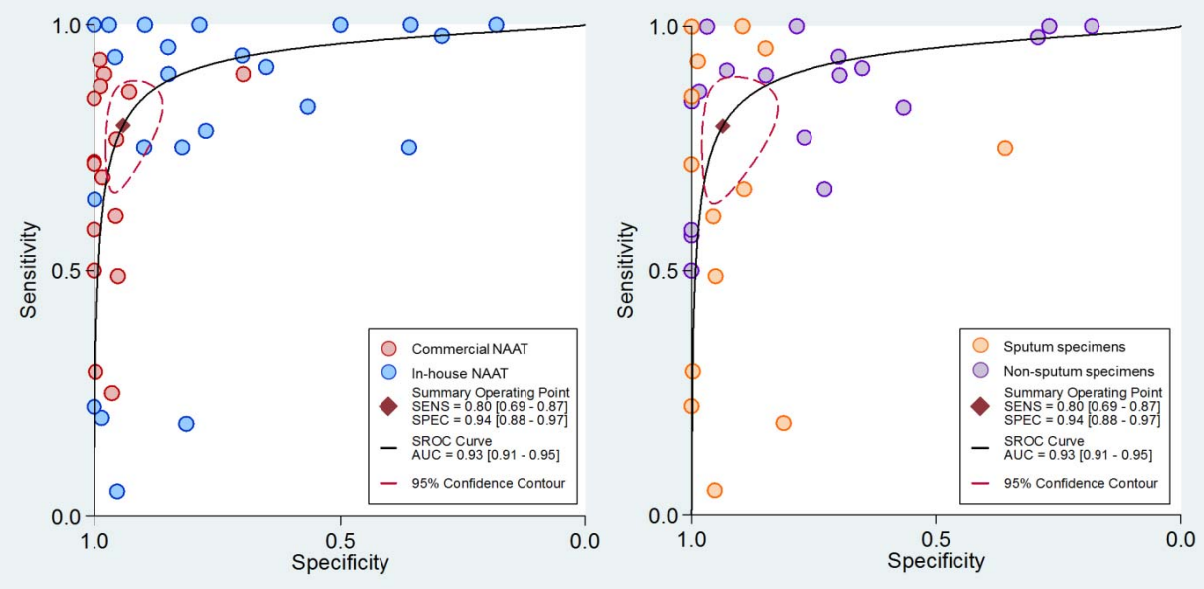

Figure 23 SROC curve for all studies investigating the sensitivity and specificity of NAAT versus culture in the diagnosis of TB for AFB-negative specimens based on NAAT methodology (A) and specimen type (B)

AUC = area under curve; NAAT = nucleic acid amplification testing; SROC = summary receiver–operator characteristic

Comparison of AFB microscopy, NAAT and AFB plus NAAT, using culture as a reference standard

Using both AFB microscopy and NAAT to diagnose MTB infections was more sensitive than using either test alone for both sputum and non-sputum specimens (Figure 24); however, there was a corresponding decrease in specificity when the two tests were combined.

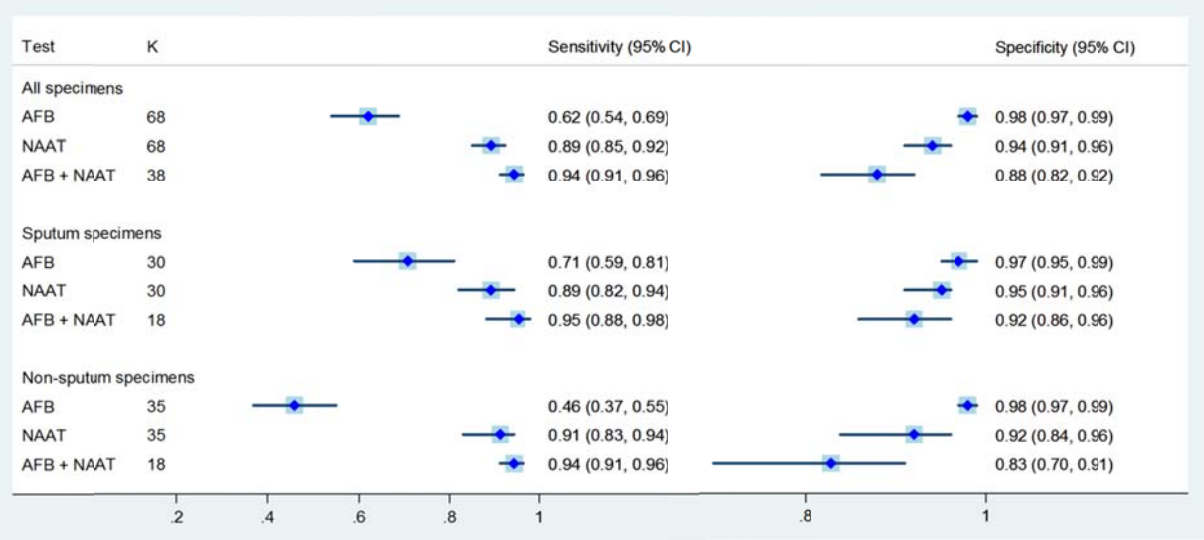

Figure 24 Forest plot showing the pooled sensitivity and specificity values for AFB, NAAT and AFB plus NAAT compared with culture according to specimen type

AFB = acid-fast bacilli; K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis.

Overall, 38% of all patients (29% of those providing sputum specimens and 54% of those providing non-sputum specimens) had a false-negative AFB microscopy result but only 2–3% were falsely positive when compared with culture. For NAAT only 11% of patients (11% with

sputum specimens and 9% with non-sputum specimens) had false-negative results and 6% (5% with sputum specimens and 8% with non-sputum specimens) false-positive results. When the two tests were combined, 5–6% of patients had a false-negative result and 12% (8% with sputum specimens and 17% with non-sputum specimens) were falsely positive. However, as not all patients with a clinical diagnosis of TB will be culture-positive, it is uncertain what proportion of these false-positive patients have been truly misdiagnosed.

The clinical impact of a higher false-positive rate will result in some patients receiving treatment for a disease they do not have, until clinical unresponsiveness is noted or culture results are available. The consequences of a false-negative result are much more severe, as the patient may remain untreated for a longer time period and could potentially spread the disease to more individuals in the community.

When the summary LR+ and LR– values were compared, some differences between the tests were observed. The LR scattergram in Figure 25 shows that the summary LR+ and LR– values for either AFB microscopy or NAAT are in the upper left quadrant. While AFB microscopy is only useful to confirm the presence of TB, NAAT also has some diagnostic value in identifying those without disease as it lies in the shaded area of this quadrant.

In contrast, when AFB microscopy and NAAT are combined the summary values are in the lower right quadrant, indicating that a negative result from both tests is a good indication that the patient will also be culture-negative for MTB. However, decreased certainty in a positive AFB microscopy or NAAT result correlating with a positive culture is due to the 22% false-positive NAAT rate for the AFB-positive population. As discussed above, culture is an imperfect reference standard; hence, many of these patients would receive a clinical diagnosis of TB.

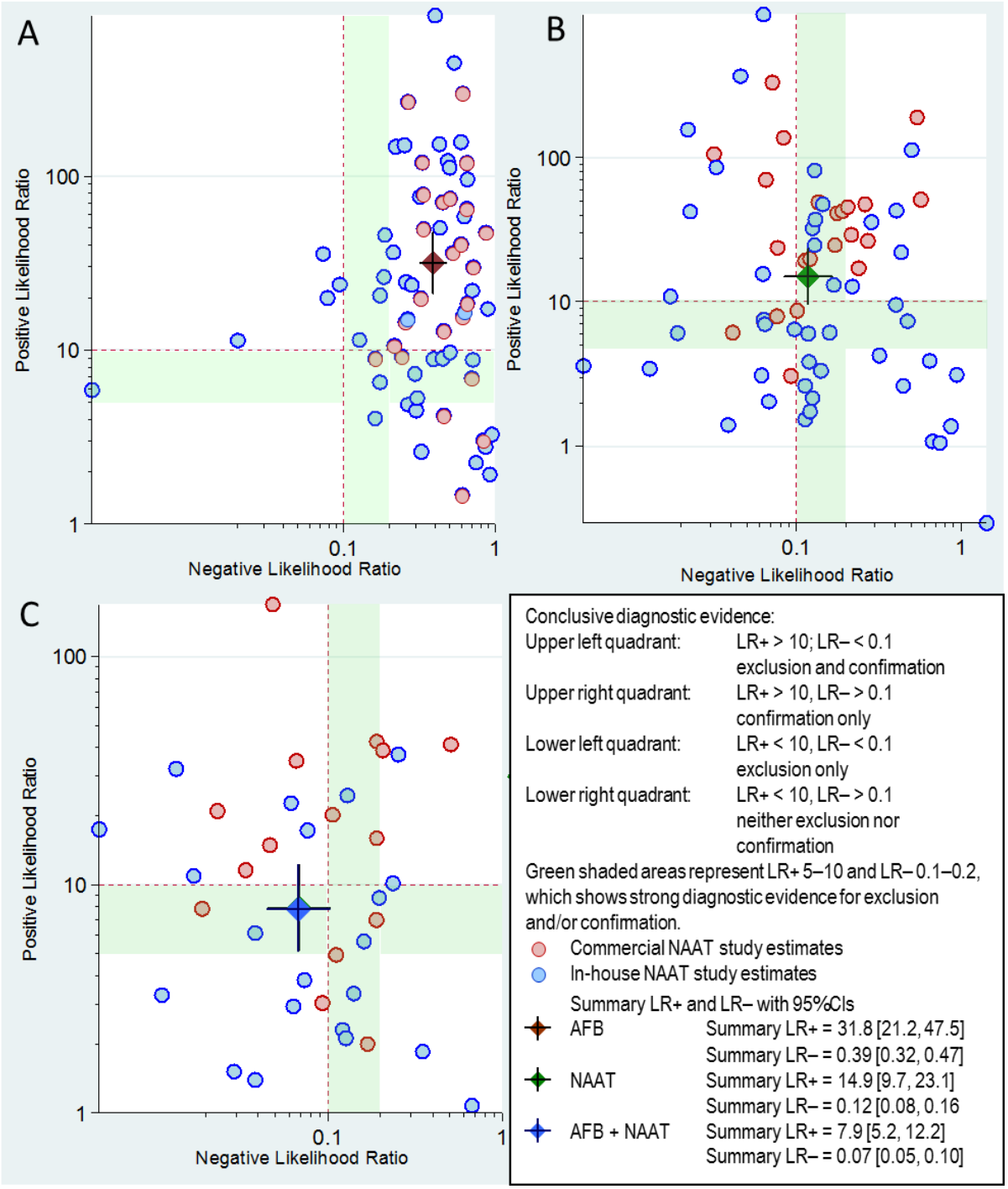

Figure 25 LR scattergram for diagnosis of MTB infection by AFB (A), NAAT (B) and AFB plus NAAT (C) compared with culture in studies using either in-house NAAT or the commercial Xpert NAAT

AFB = acid-fast bacilli; LR = likelihood ratio; NAAT = nucleic acid amplification testing

Comparison of AFB microscopy and NAAT, using culture as a reference standard in HIV-positive and HIV-negative patients

Eight studies provided data to assess the diagnostic accuracy of NAAT and AFB microscopy compared with culture in HIV-positive patients suspected of having an MTB infection. Of these, 2 studies used in-house NAAT and 6 used commercial NAAT. Five of these studies were conducted in countries with a high incidence of TB and only 2 looked at the accuracy

of NAAT in AFB-negative specimens (Figure 47 in Appendix D). Six studies provided data to assess the diagnostic accuracy of NAAT and AFB microscopy compared with culture in HIV-negative patients suspected of having an MTB infection, and all were conducted in countries with a high incidence of TB. Of these, 3 studies used in-house NAAT and 3 used commercial NAAT (Figure 48 in Appendix D). It should be noted that the *Tuberculosis notifications in Australia, 2010 Annual Report*[11] stated that HIV and TB co-infection remains rare in Australia and is a relatively minor contributor to annual TB incidence, unlike in many other parts of the world.

The pooled sensitivity and specificity values for AFB microscopy or NAAT compared with culture in HIV-positive and -negative populations were compared with those for all included studies, which largely consisted of patients in whom their HIV status was unknown (Figure 26). There were no differences between the pooled values for the three population groups, indicating that HIV status does not affect the performance of either AFB microscopy or NAAT.

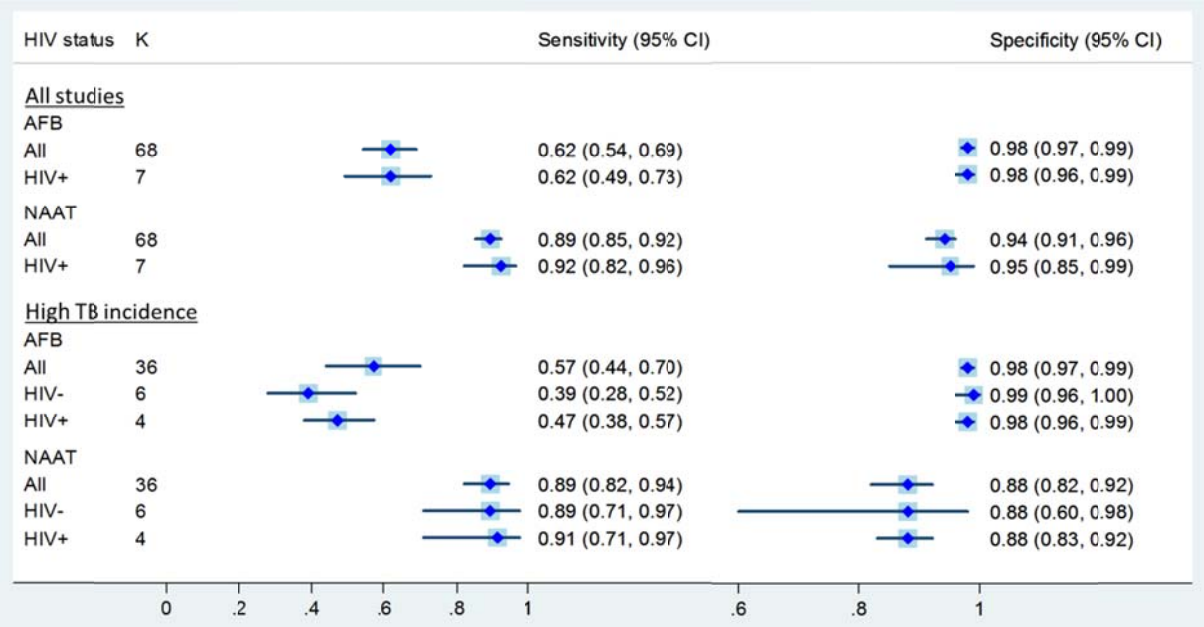

Figure 26 Forest plot showing the pooled sensitivity and specificity values for AFB and NAAT compared with culture according to HIV status

AFB = acid-fast bacilli; High TB incidence = > 100 cases per 100,000 people based on WHO estimates from 2012; HIV = human immunodeficiency virus; K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis

HIV-positive patients with pulmonary TB commonly produce AFB-negative sputum specimens (de Albuquerque et al. 2014; Scherer et al. 2011). Thus, the difficulty associated

[11]Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

with diagnosis of TB in HIV-positive patients is related to the reduced sensitivity of NAAT compared with culture in AFB-negative specimens. Figure 27 shows that 33% (1 – sensitivity) of AFB-negative sputum specimens will have a false-negative NAAT result when compared with culture; in contrast, only 3% of AFB-positive specimens will have a false-negative result. The difference in the pooled sensitivity for non-sputum specimens is more modest (14% and 0%, respectively) but still sufficient to be of some concern to clinicians.

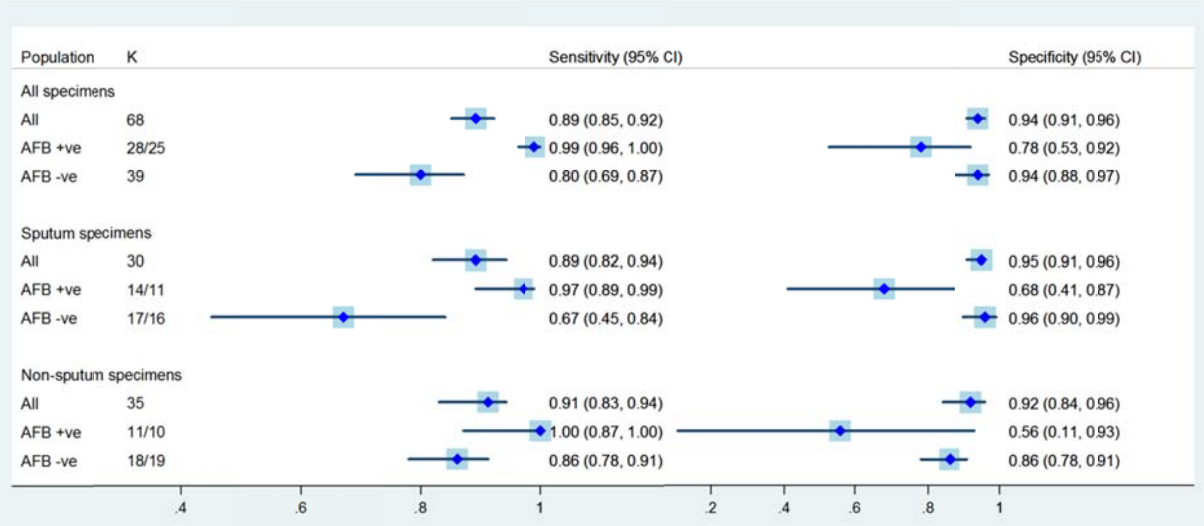

Figure 27 Forest plot comparing the pooled sensitivity and specificity values for NAAT versus culture according to AFB result and specimen type in HIV-positive specimens

AFB = acid-fast bacilli; K = the number of studies

Meta-analysis of studies assessing the diagnostic accuracy of NAAT compared with culture-based DST in detecting drug-resistant MTB infections

Eleven studies provided data to assess the diagnostic accuracy of NAAT compared with culture-based DST in patients suspected of having TB who were later found to be culture-positive (Figure 28). The prevalence of rifampicin-resistant MTB strains in these studies ranged from 0% (in 3 studies) to 63% with a median of 4% and a mean of 12%. Only 1 study used an in-house NAAT (pyrosequencing of the *rpoB* gene); the other 10 studies used the Xpert NAAT. Two of the studies also compared the Xpert NAAT to DST for the detection of MDR-MTB. In these studies detection of mutations in the *rpoB* gene was considered a surrogate measure for detecting MDR.

Meta-analysis of these studies showed that NAAT is both highly sensitive (93%; 95%CI 85, 97) and highly specific (98%; 95%CI 96, 99) compared with DST in identifying rifampicin-resistant MTB. The utility of the Xpert NAAT as a surrogate for MDR-MTB cannot be evaluated in this assessment as only 2 studies met the inclusion criteria, with vastly differing sensitivity results.

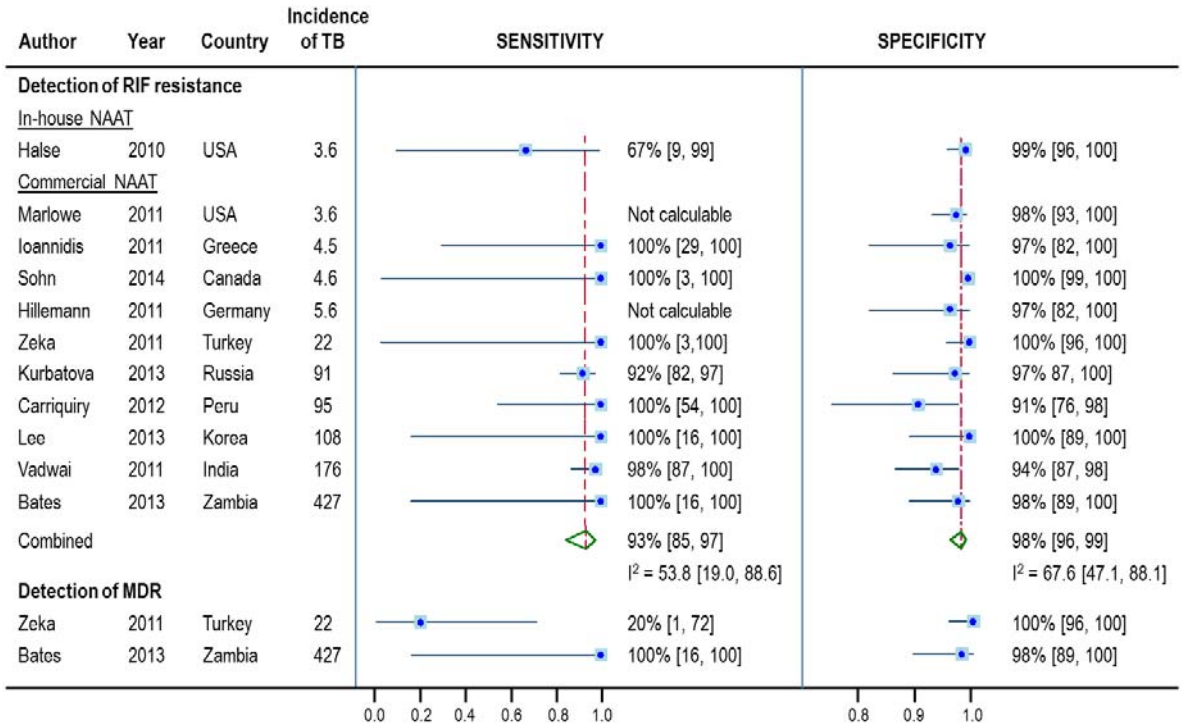

Figure 28 Forest plot of the sensitivity and specificity of NAAT compared with culture-based DST to detect drug-resistant MTB infections

MDR = multidrug resistance; NAAT = nucleic acid amplification testing; RIF = rifampicin; TB = tuberculosis

Does it change patient management?

Summary—Does NAAT change clinical management?

Seventeen relevant studies on change in management after NAAT were identified.

Not surprisingly, all studies were in agreement that the use of NAAT resulted in a quicker diagnosis of patients with TB, especially in those who were AFB-negative. Predictably, this also resulted in earlier treatment in NAAT-positive patients.

A historical control study of poor quality and a retrospective cohort study of medium quality reported that the median duration of unnecessary and/or over-treatment of TB was shorter in patients when NAAT was used to guide treatment decisions compared with those when NAAT was not available.

There were conflicting data on the likely impact of NAAT in the clinical setting. A retrospective cohort study of poor quality, conducted in the UK (medium TB incidence), concluded that clinician decision-making would be affected by NAAT results and that there would be significant clinical benefits from the use of NAAT in low-prevalence settings. Conversely, two cohort studies (one retrospective) of medium quality, conducted in Saudi Arabia (medium TB incidence) and Canada (low TB incidence), suggest that clinicians would be reluctant to change patient management based on the NAAT result.

Studies were included to assess change in management following NAAT according to criteria outlined *a priori* in Box 4.

Box 4 PICO criteria for identification of studies relevant to an assessment of change in management following NAAT in patients able to have an AFB microscopy test

Population	Patients with clinical signs and symptoms of active TB whose specimen is suitable for AFB microscopy and culture, and who have had < 3 days of anti-TB treatment Subpopulations for analysis: a. those with a high pre-test probability of active TB, e.g. come from a country with high rates of TB, versus those with a low pre-test probability of TB
Intervention	1. AFB microscopy plus NAAT for the detection of MTB-complex DNA ± culture 2. NAAT for the detection of genetic mutations on the *rpoB* gene associated with rifampicin resistance
Comparators	1. AFB microscopy ± culture 2. No NAAT for rifampicin resistance, ongoing AFB tests to determine if patient is responding to treatment
Outcomes	Time to diagnosis of TB or alternate condition, time to diagnosis of resistance, time to appropriate treatment, rate of treatment, duration of treatment, number of contacts required to be traced, number of contacts infected, rate of rifampicin resistance
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	1990 – May 2014 or inception of the database if later than 1990
Language	Studies in languages other than English were excluded unless they represented a higher level of evidence than that available in the English language evidence-base

Seventeen studies reporting change in management outcomes due to NAAT were identified. Eight of these studies were conducted in developing countries with a high TB-prevalence (e.g. South-Africa, Uganda, Peru) and 8 studies were conducted in low-prevalence countries (e.g. USA, UK, Canada). The remaining study was conducted in a country with an intermediate TB burden (Korea). Only two studies used in-house NAATs and the remainder used commercial NAAT, of which 12 used the Xpert NAAT. The study profiles are summarised in Table 97 (Appendix F) and an overall summary of the body of evidence is presented in Table 20.

Table 20 Body of evidence matrix for studies reporting change in management outcomes due to NAAT

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]		One or two level II studies with a low risk of bias, or an SR or several level III studies with a low risk of bias		
Consistency		Most studies consistent and inconsistency may be explained		
Clinical impact				Slight or restricted
Generalisability		Population(s) studied in the body of evidence are similar to target population		
Applicability			Probably applicable to Australian healthcare context with some caveats	

SR = systematic review; several = more than two studies

[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).

Source: Adapted from NHMRC (2009)

Time to diagnosis and/or treatment

Fourteen studies reported data on time to TB diagnosis or anti-TB treatment after the intervention/comparator. Eight of these studies were conducted in countries with relatively high TB prevalence and 1 with intermediate TB prevalence (Table 97 in Appendix F). Median time to TB diagnosis and median time to therapy are shown in Table 21 and Table 22, respectively. It was shown that time to diagnosis is shorter with NAAT, compared with liquid and solid culture, and similar to AFB microscopy. Median time to therapy is also decreased with the use of NAAT (in these cases, Xpert) compared with other methods of diagnosis (especially culture), as shown in Table 22. These results correspond with those from two other studies: (1) a prospective cohort study by Sohn et al. (2014), which stated that for five subjects in their study who had AFB-negative Xpert-positive results, treatment would have started a median of 12 days (IQR 4–23) earlier if results had been shared with the physicians, whereas treatment would have been only around 1 day sooner for AFB-positive cases; and (2) a Spanish retrospective cohort study by Buchelli Ramirez et al. (2014), which reported that in the sputum AFB-negative group, Xpert-positive results allowed for an early treatment start. In this study treatment was brought forward by 26.1 ± 14.5 days, without waiting for culture results.

In addition to the median time to diagnosis and treatment data, a historical control study by Yoon et al. (2012) reported that the proportion of TB patients diagnosed on day 1 using AFB microscopy was 55%, compared with 78% in patients diagnosed by AFB microscopy plus Xpert NAAT ($p<0.001$). The study by Theron et al. (2014) showed that 44% (67/154) of culture-positive patients in a group that had AFB microscopy started treatment on the day of presentation, compared with 66% (122/170) in a group that had Xpert NAAT ($p<0.0001$). Furthermore, a medium-quality retrospective cohort study by Kwak et al. (2013) reported that the median turnaround time for Xpert results in Korea was 0 days (IQR 0–1), which was significantly less than AFB microscopy with a turnaround time of 1 day (IQR 0–1), liquid or solid culture with 14 days (IQR 10.25–1.75) and 24 days (IQR 17–30) respectively, and DST with 78 days (IQR 65–96). Time to confirmation of results by a physician was also significantly shorter for Xpert results in this study, with a median of 6 (IQR 3–7) days, compared with 12 (IQR 7–19.25), 21 (IQR 7–19.25), 38.5 (IQR 25.75–50.25) and 90 (IQR 75.75–106) days for AFB microscopy, liquid culture, solid culture and DST, respectively. Median turnaround time (from sampling to reporting) was also reported in the retrospective study by Omrani et al. (2014), which was 1 day for Xpert NAAT, 1 day for AFB microscopy ($p>0.999$) and 44 days for mycobacterial cultures ($p<0.001$). Laboratory processing times for

AFB microscopy were 2.5 times as long as Xpert NAAT (23.2 hours, IQR 15.3–32.6 versus 9.1 hours, IQR 5.5–15.6, p<0.001), as stated by a cohort study done in the US (Lippincott et al. 2014).

Table 21 Median time to TB diagnosis/detection using NAAT versus comparator

Study Country	Number of patients	Median time to TB diagnosis, NAAT (IQR)	Median time to TB diagnosis, comparator (IQR)
Boehme et al. (2010) Peru, South Africa, Uganda, Philippines	N=6648	Xpert: 0 days (0–1)	AFB microscopy: 1 day (0–1) Solid culture: 30 days (23–43) Liquid culture: 16 days (13–21)
Sohn et al. (2014) Canada	N=502	Xpert: 25 hours (3–39)	AFB microscopy: 26 hours (25–51) Culture: 516 hours, 22 days (336–720)
Van Rie et al. (2013b) South Africa	N=344	Xpert: 1 day (1–1)	AFB microscopy: 8 days (5–10) Culture: 29 days (24–35)
Fan et al. (2014) China	N=280	NAAT (in-house): 0.5 day	Liquid culture: 28.2 days (15–50) (p<0.001)
Yoon et al. (2012) Uganda	Baseline N=157 Implementation (Xpert) N=105	Xpert: 0 days (0–1), range 0–55	AFB and/or microscopy: 1 day (0–26)

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF assay

Table 22 Median time to therapy using GeneXpert versus comparator

Study	Number of patients	Median time in days to therapy, NAAT (IQR)	Median time in days to therapy, comparator (IQR)	p-value
Boehme et al. (2010) Peru, South Africa, Uganda, Philippines	N=6648	Xpert in AFB-negative, culture-positive TB: 5 (2–8)	AFB-negative, culture-positive TB (other methods): 56 (39–81)	-
Hanrahan et al. (2013) South Africa	Xpert positive N=50 Empiric TB N=25 X-ray positive N=19 Culture positive N=20	Those positive on Xpert: 0	Empiric TB: 14 (5–35) Suggestive chest X-ray: 14 (7–29) Culture positive (Xpert negative): 144 (28–180)	-
Kwak et al. (2013) Korea	Xpert N=43 No Xpert N=86	Xpert: 7 (4–9)	No Xpert (culture and/or AFB): 21 (7–33.5)	<0.001
Omrani et al. (2014) Saudi Arabia	Xpert N=76 Comparator N=64	Xpert: 0	AFB microscopy: 0 Culture: 22	>0.999 <0.001
Theron et al. (2014) South Africa	Microscopy N=758 Xpert N=744	Xpert: 0 (0–3)	AFB microscopy: 1 (0–4)	0.0004
Van Rie et al. (2013b) South Africa	N=344	Xpert: 1 (1–1) N=162	Other methods [a]: 8 (1–42)	
Van Rie et al. (2013a) South Africa	N=160	Xpert: 0 (0–0)	Other methods: 13 (10–20)	<0.001
Yoon et al. (2012) Uganda	Baseline N=157 Implementation (Xpert) N=105	Xpert: 0 (0–2)	AFB microscopy: 1 (0–5)	0.06

[a] Xpert-negative, culture-positive participants (N=10); six patients started treatment before the culture result was available.
AFB = acid-fast bacilli; IQR = interquartile range; MDR = multi-drug resistant; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF assay.

The RCT by Theron et al. (2014) also reported the proportion of patients initiating TB treatment and the reasons for treatment initiations, as shown in Table 23 and Figure 29. The proportion of patients receiving treatment (regardless of reason of initiation) was higher in the Xpert group than in the AFB microscopy group during days 1–9. Furthermore, there were more culture-positive patients on treatment in the Xpert group, compared with the AFB microscopy group, until day 56.

Table 23 Proportion of patients initiating treatment based on AFB microscopy or NAAT results, by day

By day:	Positive AFB microscopy (%)	Positive NAAT (%)
1	67/758 (9%)	130/744 (17%)
2	99/758 (13%)	170/744 (23%)
3	105/758 (14%)	172/744 (23%)
14	105/758 (14%)	181/744 (24%)
28	111/758 (15%)	181/744 (24%)
56	111/758 (15%)	182/744 (24%)

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test
Source: Theron et al. (2014)

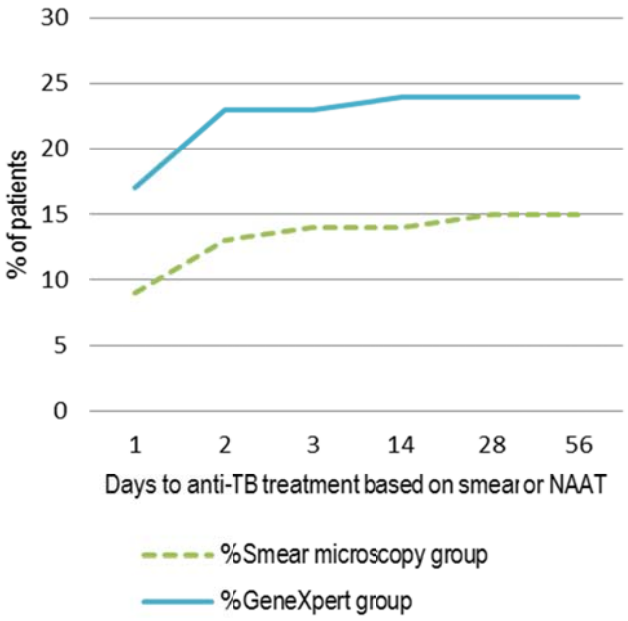

Figure 29 Percentage of patients initiating treatment based on smear (AFB microscopy) or NAAT results, by day
Source: Theron et al. (2014)

Regarding antibiotic resistance detection, Boehme et al. (2010) reported that the median time for rifampicin resistance results was 1 day (IQR 0–1) for the Xpert NAAT, compared with 20 days (IQR 10–26) for line probe assay and 106 days (IQR 30–124) for phenotypic susceptibility testing.

Time-related management results other than median time to diagnosis/treatment after Xpert NAAT were reported in three studies (Lacroix et al. 2008; Marks et al. 2013;

Taegtmeyer et al. 2008), all in low-prevalence countries The poor-quality retrospective cohort study by Lacroix et al. (2008) showed that the average delay in TB diagnosis in a public health department in Quebec was decreased by the use of PCR (n=77) compared with no use of PCR (n=38), with a mean of 89.3 days (95%CI 76.4, 102.2) compared with 97.9 days (95%CI 74.2, 121.6; p=0.498), respectively. NAAT (MTD, Gen-Probe, San Diego, California) also significantly decreased the average time to final TB determination for all patients except for those with AFB-negative NAAT-positive culture-negative specimens versus patients with AFB-negative culture-negative (no NAAT) specimens in an unadjusted analysis by Marks et al. (2013). Furthermore, this medium-quality retrospective cohort study, conducted in the USA, reported a multivariable analysis of time to determination of AFB-positive culture-positive patients, and found that a NAAT result reduced the time to TB diagnosis (adjusted hazard ratio = 2.3; 95%CI 1.4, 3.7). A different medium-quality retrospective cohort study (UK) used a INNO-LiPA RIF TB assay (Immunogenetics, Zwijndrecht, Belgium) and compared the mean time to identification of MTB and rifampicin resistance with AFB microscopy and/or mycobacterial culture (Taegtmeyer et al. 2008). The mean time to detection of mycobacteria and rifampicin resistance was 8.8 ± 5.9 days with NAAT compared with 26.0 ± 10.9 days to identification (p=0.001) using culture (without NAAT). In this study, for all the AFB-positive samples, NAAT identified 86% of the samples within 2 weeks, compared with only 7% of samples using culture.

Thus, all studies were in agreement that the use of NAAT resulted in a quicker diagnosis of patients with TB, especially in those who were AFB-negative. Predictably, this also resulted in earlier treatment in NAAT-positive patients.

Impact on TB treatment

Unnecessary treatment and overtreatment for TB were reported in three studies. Forty-seven out of 143 patients without culture-positive TB were initially treated empirically pending culture results in the medium-quality cohort study by Davis et al. (2014). Xpert results were negative in 45/47 (95.7%) of these patients, whereas 1 patient who was not treated had a positive result. Only 8 (18%) of these 45 patients were clinically diagnosed with culture-negative TB. In conclusion, 82% (37/45) of patients correctly classified by Xpert were over-treated for active TB. If Xpert had been used to guide initial treatment decisions, the median duration of overtreatment would have been 1 day (IQR 1–3) compared with 46 days (IQR 45–49), a median difference of 44 days (IQR 43–47). In this scenario 44 fewer patients would have started empirical TB treatment, and during the 13-month study period the total number of overtreatment days would have decreased by 95%, from 2,280 (95%CI 2,081, 2,479) to 111 (95%CI 0, 256) days.

Although these results were hypothetical, they correspond with results from a historical control study of poor quality by Guerra et al. (2007), which reported a median duration of non-indicated TB treatment of 6 days for patients undergoing NAAT (MTD; Gen Probe, San Diego, CA) and 31 days for the non-NAAT group (p=0.002). Furthermore, a retrospective cohort study of medium quality by Marks et al. (2013) reported culture-negative NAAT-negative patients had significantly fewer average days on outpatient medications, compared with those receiving no NAAT, with an average of 3 versus 57 days for AFB-positive culture-negative patients and 58 versus 100 days for AFB-negative culture-negative patients, respectively ($p<0.05$).

Conversely, a retrospective cohort study of medium quality from Saudi Arabia (mediun TB incidence of 15/100,000 people[12]) reported a lack of change in overtreatment and patient management after NAAT in current clinical practice (Omrani et al. 2014). Anti-TB therapy was not discontinued in any patients with negative Xpert results that started therapy empirically (n=8). Furthermore, Xpert was requested in only 54.3% (76/140) of patients and, overall, an Xpert-positive result was the reason for therapy initiation in just 12.1% (17/140) of patients. The authors concluded that physicians who are highly experienced in the diagnosis and treatment of TB underused the Xpert NAAT and it had only a limited impact on their decisions related to starting or stopping anti-TB therapy.

A cohort study of medium quality by Sohn et al. (2014), conducted in Canada (low TB incidence of 4.6/100,000 people), also reported that the Xpert NAAT had no impact in preventing unnecessary TB treatment; however, in these patients species confirmation was done by existing NAAT in the clinical lab within a day of the positive AFB microscopy, and clinical suspicion in these cases was low.

NAAT had a clinical impact on management in 39% (20/51, 95%CI 27%, 53%) of patients in the retrospective cohort study of poor quality by Taegtmeyer et al. (2008), conducted in the UK (medium TB incidence of 15/100,000 people). In 7 patients for whom there was uncertainty about TB diagnosis, TB was confirmed by NAAT and TB therapy continued. Three patients who had started empiric TB treatment were able to stop because of NAAT results, 2/4 patients with MRD-TB were identified by NAAT, and in 5 patients (previously treated) MDR-TB was excluded. In 3 patients the need for a hospital contact-tracing exercise was confirmed. The study further hypothesised that if NAAT had been used in the other 36 patients for whom it was indicated, it could have had a clinical impact on 8 of them (22%). If

[12] World Health Organization (WHO) estimates of tuberculosis incidence, 2012 (WHO 2013)

NAAT had been used in the 36 patients who were AFB-positive (not indicated by British guidelines), the results could have stopped unnecessary treatment in 14% (5/35, 95%CI 5%, 29%) of patients who did not have TB, provided rapid confirmation in 19 patients and excluded TB in a further 12 patients. The authors concluded that there were significant clinical benefits from the use of NAAT in low-prevalence settings.

Does change in management improve patient outcomes?

Summary—Do alterations in clinical management and treatment options have an impact on the health outcomes of patients diagnosed with TB?

Early versus delayed treatment of TB

Two prospective cohort studies of poor quality reported that a delay in time to diagnosis, defined as the period from onset of any TB symptoms to the diagnosis of TB, was significantly associated with an increased risk of transmission of infection among contacts. A retrospective cohort study of poor quality, conducted in New Zealand, indicated that the time between development of symptoms and diagnosis was not significantly associated with the odds of achieving a favourable treatment outcome (i.e. cure or treatment completed).

Early identification of drug resistance

No studies were identified that met the PICO criteria. However, three cohort studies (two retrospective) of medium quality provided some evidence that patients with rifampicin-resistant TB who received a rifampicin-containing Category II treatment, before receiving the results of drug sensitivity testing, had slightly poorer health outcomes than those who did not.

Unnecessary antibiotic treatment

All TB patients are at risk of adverse health events (e.g. hepatitis) associated with first-line treatment. Two SRs, one of medium quality and one of poor quality, found that some but not all AEs as a consequence of patients with active TB receiving inappropriate antibiotic treatment (due to MTB resistance) may be avoided with appropriate treatment, to which the MTB strain is sensitive. One SR of good quality found that patients have a higher risk of developing multidrug-resistant TB (MDR-TB) if they receive inappropriate drug treatment.

To answer the question whether a change in management leads to improved patient health outcomes, additional literature searches were done based on the PICO criteria outlined *a priori* in Table 24.

Table 24 PICO criteria for identification of studies relevant to an assessment of health outcomes following a change in management

	Early versus delayed treatment of TB	Early identification of drug resistance	Unnecessary antibiotic treatment
Population	Patients with clinical signs and symptoms of active TB and a low pre-test probability of active TB	Patients with clinical signs and symptoms of active TB and a high pre-test probability of active TB, who are identified at some point as having rifampicin resistance Subgroups: those patients able to have an AFB microscopy, and those unable to have AFB microscopy	Patients with clinical signs and symptoms of active TB (true positives or false positives)
Intervention	Immediate treatment for TB, i.e. antibiotics	Early treatment with antibiotics, other than rifampicin	Treatment for TB with antibiotics
Comparators	Treatment for TB delayed 6–8 weeks (due to negative AFB result, negative NAAT result, or clinical judgement that patient does not have TB based on histology, until culture results received)	Standard treatment for active TB (including rifampicin), delayed treatment with alternative antibiotics	No treatment for TB
Outcomes	Time to symptom resolution, quality of life, length of infectious period, number of contacts infected	Time to symptom resolution, quality of life, length of infectious period, number of contacts infected	AEs from antibiotic treatment
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs	Randomised trials, cohort studies, case series or systematic reviews of these study designs	Systematic reviews of randomised trials
Search period	1990 – June 2014 or inception of the database if later than 1990	1990 – June 2014 or inception of the database if later than 1990	1990 – June 2014 or inception of the database if later than 1990
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base

What health impact does early versus delayed treatment of TB have on the individual and their contacts?

Delay in treatment of TB resulting from failure to diagnose TB prior to the availability of culture results is likely to prolong the duration of symptoms in the patient, with a corresponding reduction of quality of life (QoL). In addition, treatment delay increases the duration of exposure for those individuals in contact with the patient, with a corresponding increase in the risk of transmission (American Thoracic Society 1992).

None of the articles identified by the literature search to identify evidence regarding the health implications for both the patient and their contacts resulting from a delay of treatment of active TB of a duration consistent with the time required to obtain culture

results (6–8 weeks) completely fulfilled the PICO criteria outlined in Table 24. However, three studies provided evidence of reasonable relevance to this section of the report. Ponticiello et al. (2001) and Golub et al. (2006) examined the effect of a delay in diagnosis of TB on the risk of infection among the close contacts of the patient, while van der Oest, Kelly & Hood (2004) assessed the effect of delay on patients' treatment outcomes. The study profiles are summarised in Table 98 (Appendix F) and an overall summary of the body of evidence is presented in Table 25.

Table 25 Body of evidence matrix for studies assessing the health impact of early versus delayed treatment of TB

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]				Level IV studies, or level I to III studies/SRs with a high risk of bias
Consistency		Most studies consistent and inconsistency may be explained		
Clinical impact				Slight or restricted
Generalisability		Population(s) studied in the body of evidence are similar to target population		
Applicability		Applicable to Australian healthcare context with few caveats		

SR = systematic review
[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).
Source: Adapted from NHMRC (2009)

Ponticiello et al. (2001) presented the results of a poor-quality prospective cohort study of TB patients and their close contacts to investigate sociodemographic and clinical risk factors for transmission of TB, including delay in diagnosis (Table 26). The study was conducted at a TB referral centre in Italy, and included patients with newly diagnosed pulmonary TB during the period January 1997 and December 1998, and their close contacts. Close contacts included the patient's household and all other persons sharing the same indoor environment with the patient for prolonged periods. The applicability of this study is limited by the fact that the effect of extended delay to diagnosis was compared with a diagnostic delay of up to 1 month; the risk of transmission to contacts when treatment was initialised immediately upon development of symptoms was not reported. In addition, delay in diagnosis was defined as the period from onset of any TB symptoms to the diagnosis of TB, which includes both the patients' delay in seeking medical attention (presumably as reported by the patient) as well as health-system delays in diagnosis and treatment initiation.

All the patients included in the study had AFB microscopy of sputum or bronchial specimens. The average delay in diagnosis of TB was 2.25 ± 1 month. In a multivariate logistic regression model, delay time to diagnosis was the only factor that remained significantly and independently associated with an increased risk of infection among contacts, as determined by positivity to the tuberculin skin test ($p<0.0002$). For patients with a diagnostic delay of 1.5 months the adjusted odds ratio for contacts infected / not infected, compared with a diagnostic delay of less than 1 month, was 4.2 (95%CI 1.3, 13.7), while with a diagnostic delay of 2 months this increased to 6.1 (95%CI 1.9, 19.6).

Golub et al. (2006) performed a similar prospective cohort study of poor quality to determine the association between total treatment delay and TB transmission in patients with verified pulmonary TB reporting to the Maryland Department of Health and Mental Hygiene in the USA between June 2000 and November 2001 (Table 26). Total treatment delay was defined as the interval from first TB symptoms to initiation of treatment for TB. The median total treatment delay for US-born patients was 99 days, with 67% (36/54) of patients having delays ≥ 60 days. The probability of having infected contacts (tuberculin skin test positive) increased with longer delay in diagnosis; 38% of contacts of patients with a delay of ≥ 60 days had positive tuberculin skin tests compared with 25% of contacts with < 60 days' delay ($p=0.05$). Although these results are not surprising, they reinforce the belief that quicker diagnosis of TB is of great benefit in reducing its spread to close contacts of infected individuals.

van der Oest, Kelly & Hood (2004) performed a retrospective study of poor quality based on notified cases of TB among residents of the Waikato Health District, located in the North Island of New Zealand, from January 1992 to December 2001 (Table 26). Although the outcome measure used in the study—favourable treatment outcome (i.e. cure or treatment completion)—was not specified in the PICO, due to the paucity of relevant evidence on the health impact of treatment delay on the patient, this study was included. The definition of diagnostic delay was the time between development of symptoms and diagnosis. While 84.7% of patients were reported as having delayed diagnosis of over 4 weeks, the mean and standard deviation of the time to diagnosis were not provided. Of those patients for whom data were available, 79% successfully completed treatment. The results of a logistic regression model indicated that time between development of symptoms and diagnosis was not significantly associated with a favourable treatment outcome (OR=1.02; 95%CI 0.99, 1.04; $p=0.15$). As 'favourable treatment outcome' was poorly defined in this study, this result may simply reflect that the treatment completion rate, which may be influenced by many factors, appears to be unrelated to any treatment delays.

Table 26 Summary of studies assessing the health impact of early versus delayed treatment of TB on the individual and their contacts

Study	Definition of treatment delay	Results
Effect on contacts		
Ponticiello et al. (2001) Italy N=90 source cases, 227 contacts	Delay defined as period from onset of any TB symptoms to diagnosis Reference: delay to diagnosis ≤ 1 month	Prevalence of TST+ among contacts (all cases) 125/227 (45%: 39–51%) 18/125 (14%) developed active TB % contacts TST+ by treatment delay 1 month 6/43 (14.0%) (reference) 1.5 months 15/37 (40.5%) 2 months 24/56 (42.9%) 2.5 months 13/23 (56.5%) OR_{adj}: TST+/TST– for long delay versus TST+/TST– for 1-month delay, (95%CI) [a] 1.5 months OR_{adj}=4.2 (1.3, 13.7) 2 months OR_{adj}=6.1 (1.9, 19.6) 2.5 months OR_{adj}=7.5 (1.9, 30.3)
Golub et al. (2006) USA N=54 US-born patients, 310 contacts	Interval from first TB symptoms to initiation of treatment Delay treated as dichotomous variable with cut-off of either 60 days or 90 days Median total delay 99 days	Patients with treatment delay: ≥ 60 days 36/54 (67%) ≥ 90 days 30/54 (56%) Contacts TST+ (delay 60 days) p=0.05 [b] Delay < 60 days 18/71 (25%) Delay ≥ 60 days 91/239 (38%) Contacts TST+ (delay 90 days) p<0.01 [b] Delay < 90 days 24/100 (24%) Delay ≥ 90 days 85/210 (40%) Total delay ≥ 90 days as predictor of TST + contacts (GEE multivariate model) OR_{adj}: 2.34 (95%CI 1.07, 5.12) p=0.03 TB cases among contacts Delay 46–66 days 2/10 Delay ≥ 90 days 8/10
Clinical effect on patient		
Van der Oest, Kelly & Hood (2004) New Zealand N=244 Patients with documented length of delay=152 (62%)	Delay between development of symptoms and notification of the case	Favourable treatment outcome OR: 1.02 (95%CI 0.99–1.04) p=0.15

CI = confidence interval; OR = odds ratio; OR_{adj} = adjusted odds ratio; TST = tuberculin skin test

[a] Adjusted OR, determined from logistic regression

[b] Favourable treatment outcome as defined by the WHO: cure or treatment completed

[c] OR determined from logistic regression

In summary, 2 studies reported that a delay in time to diagnosis was significantly associated with an increased risk of transmission of infection among contacts. Although these results are not surprising, they reinforce the belief that quicker diagnosis of TB is of great benefit in reducing the spread of TB to close contacts of infected individuals. The lack of an effect on favourable treatment outcomes after earlier initiation of treatment found in a retrospective

cohort study agrees with the findings of the 2 studies, providing direct evidence on the effect of including NAAT in clinical decision-making. Those 2 studies found that inclusion or exclusion of NAAT results had no effect on morbidity and mortality rates.

To what extent does treating patients who have rifampicin-resistant MTB infections with alternative treatments result in better health outcomes for the patient and their contacts?

The aim of this literature search was to determine the effectiveness of change in management due to rifampicin-resistance mutations being identified. This could impact mortality, time to symptom resolution, QoL, the length of the infectious period, or the number of contacts infected with TB. None of the articles found completely met the PICO criteria found in Table 24. However, 3 studies provided some evidence to answer part of the research question. The study profiles can be found in Table 99 (Appendix F) and an overall summary of the body of evidence is presented in Table 27.

Table 27 Body of evidence matrix for studies investigating the effect of change in management due to detection of drug resistant MTB

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]				Level IV studies, or level I to III studies/SRs with a high risk of bias
Consistency [b]	All studies consistent			
Clinical impact				Slight or restricted
Generalisability			Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population	
Applicability		Applicable to Australian healthcare context with few caveats		

SR = systematic review
[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).
Source: Adapted from NHMRC (2009)

A study by Meyssonnier et al. (2014) reported the outcomes of a retrospective cohort of rifampicin mono-resistant TB patients in France. At the time of TB diagnosis 83% (25/30) received rifampicin-containing regimens (Table 28). The remaining 5 patients did not receive rifampicin due to suspected resistance because of a previous treatment history or the first-line DST results from other countries or contacts. However, when DST results to first-line drugs were available in the study population, 3 patients did not have any modification of the rifampicin-containing regimen. Two of these patients were considered cured after 9 months

of treatment and a 2-year follow-up (2 months of standard four-drug regimen and 7 months of rifampicin and isoniazid). The third patient died after 9 months of standard treatment, but also had a Kaposi sarcoma related to HIV co-infection. Of the patients receiving antibiotic treatment other than rifampicin, 13 (52%) received fluoroquinolone-containing regimens without aminoglycoside, 4 (16%) received amikacin-containing regimens without fluoroquinolone and 8 (32%) received both fluoroquinolones and amikacin.

Table 28 Association between treatment characteristics and health outcomes among rifampicin-resistant TB patients

Health outcomes	RIF containing regimen (n=3)	Antibiotics, other than RIF (n=25)
Recovery	2 (67%)	16 (64%)
Lost to follow-up	–	3 (12%)
Dead	1 (33%)	3 (12%)
Relapse	–	3 (12%)

RIF = rifampicin
Source: Meyssonnier et al. (2014)

The second study, a Thai retrospective chart review by Lam et al. (2014), identified that patients with rifampicin-resistant or MDR-TB who received rifampicin-containing Category II treatment (streptomycin, isoniazid, ethambutol, rifampicin, and pyrazinamide) before DST results had poorer treatment outcomes than those who received the treatment post-DST (Table 29). Patients who completed treatment and those who were cured of TB were considered to have successful outcomes; patients for whom treatment failed and those who defaulted or died were considered to have poor treatment outcomes. However, it was not reported which combination of antibiotic treatments the 'no rifampicin' group received (possibly variable).

Table 29 Association between treatment characteristics and poor treatment outcome among rifampicin-resistant and MDR-TB patients, Thailand 2004–08

Category II treatment	Poor outcome / total outcomes	Univariate OR [95%CI]	p-value	Multivariate OR [95%CI]	p-value
None	50/155 (32.3%)	Reference		Reference	
Pre-DST only	15/26 (57.7%)	2.9 [1.2, 6.7]	0.02	2.5 [1.1, 6.4]	0.05
Post-DST / full treatment course	4/9 (44.4%)	1.7 [0.4, 6.5]	0.45	2.8 [0.7, 11.6]	0.16

CI = confidence interval; DST = drug susceptibility testing; OR = odds ratio
Source: Lam et al. (2014)

The third study, a British cohort study by Drobniewski et al. (2002), showed survival curves comparing MDR-TB patients treated with three drugs to which the bacterium was susceptible on DST results and those treated with fewer agents, also with demonstrable susceptibility. In the first group (n=62) the median survival period was 2,066 days or 5.66 years (95%CI 1336, 2515), and in the second group (n=13) this was 599 days or

1.64 years (95%CI 190, 969). Although this study did not meet the PICO criteria for inclusion, the authors reported that those who received appropriate treatment would have a longer median survival time and a lower chance of death, with an estimated risk ratio of 0.06 (95%CI 0.01, 0.23). Furthermore, those in whom culture results were available within 30 days were less likely to die, with a risk ratio of 0.23 (95%CI 0.06, 0.86), indicating the importance of accurate DST data in the clinical management of patients.

These 3 studies suggest that patients with rifampicin-resistant TB who receive rifampicin-containing anti-TB regimens have a slightly worse prognosis than those who receive other regimens. However, the reports are of limited applicability as they did not meet the PICO criteria.

What are the AEs associated with unnecessary antibiotic treatment?

A likely outcome of a change in first-line management of patients with active TB, specifically those who receive antibiotic treatment inappropriate for the genotype (and resistance) of the TB bacillus causing their disease, is the AEs and reactions associated with their antibiotic therapy. A literature search was conducted to identify evidence regarding the safety of first-line antibiotic treatment in patients with active TB. The PICO criteria for identification of the literature can be seen in Table 24. Evidence was sought for the first-line drugs isoniazid, rifampicin, ethambutol, myambutol and pyrazinamide. Due to the volume of articles identified, only the highest level of evidence is included here.

Three SRs were included (Forget & Menzies 2006; Frydenberg & Graham 2009; van der Werf et al. 2012). Study profiles for the three SRs can be found in Table 100 in Appendix F, and an overall summary of the body of evidence is presented in Table 30.

Table 30 Body of evidence matrix for studies assessing the health impact of inappropriate antibiotic treatment

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]	One or more level I studies with a low risk of bias or several level II studies with a low risk of bias			
Consistency [b]	All studies consistent			
Clinical impact			Moderate	
Generalisability			Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population	
Applicability		Applicable to Australian healthcare context with		

		few caveats		

SR = systematic review; several = more than two studies
[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).
Source: Adapted from NHMRC (2009)

The review by Forget and Menzies (2006), assessed as medium quality, searched only one database (Medline) and in addition sought relevant articles from the authors' files and pearled references. While the review did not meet all criteria that define an SR, it nevertheless provided the best identified evidence across a range of first-line drugs for prophylactic treatment in patients of all ages with active TB and. A second review of medium quality assessed the toxicity of first-line drugs and prophylactic treatment in children with TB through literature identified in PubMed, EMBASE and the Cochrane Library Reference (Frydenberg & Graham 2009). In addition, reference lists were hand-searched for relevant articles. The third relevant study identified assessed evidence of multidrug resistance following inappropriate TB treatment, and was of high quality (van der Werf et al. 2012). This study conducted a broad systematic literature search and used clearly defined criteria for appropriate treatment regimens and multidrug resistance.

Adverse reactions to first-line TB therapy (all ages)

Forget and Menzies (2006) reported on the serious adverse reactions associated with five first-line anti-TB drugs. Their results for isoniazid, rifampicin, pyrazinamide and ethambutol will be discussed here. The authors comment that the attribution of side effects to individual drugs is challenging as most patients are given multidrug regimens; however, if a temporal relationship between a drug and symptoms could be established, the symptoms were attributed to that drug. The authors of this review used the attribution made by the primary study authors. In some studies attribution of symptoms could only be made to the treatment regimen, and therefore the adverse reactions to multidrug TB regimens are also reported briefly here.

Isoniazid

Metabolism of isoniazid therapy is dependent on two pathways (direct and indirect) associated with different levels of activity in patients who have either high or low N-acetyltransferase activity (i.e. fast or slow acetylators). N-acetyltransferase activity is associated with race (90% of Asian, 45% of black, and 45% of white people are fast acetylators) and hepatic adverse reactions (Mitchell et al. 1975; Mitchell et al. 1976). A summary of hepatic adverse reactions reported in 10 studies that administered isoniazid as chemoprophylaxis against TB is tabulated in Table 31.

Table 31 Summary of hepatic AEs to isoniazid from prospective and retrospective studies

Systematic review Included studies	Total participants (N)	Total cases (N)	Adjusted rate per 1,000	Adjusted mortality per 1,000
Forget and Menzies (2006) k=10	64,278	399	9.2	0.43

Forget and Menzies (2006) found that there was heterogeneity between these 10 studies, although they did not provide a statistical summary. In particular, the definition of a hepatic AE varied between studies; for example, the definition of 'hepatitis' ranged from asymptomatic elevation in liver enzymes to clinical hepatitis. Study populations varied in the proportion of female participants (45.6% to 100% in studies that reported this factor) and in age. A prospective study in children (mean age 11 years) reported a low case rate of 11.6 per 1,000 when compared with a retrospective cohort study in which participants had a mean age of 76 years and reported a case rate of 49.9 per 1,000; however, both articles reported a mortality rate of zero.

Nine of the 10 studies reported completion/compliance rates at 12 months following initiation of treatment. Rates ranged from 16%/46% (completion/compliance at 6 months) in a retrospective cohort of 3,681 female patients who began therapy during pregnancy, to 95% completion in the prospective study of 434 children previously mentioned. The cohort of pregnant females also reported the highest adjusted mortality rate[13] (1.18 per 1,000). Four of the 10 studies reported adjusted mortality rates of 0 per 1,000.

Incidence of hepatic AEs (adjusted rate per 1,000)[14] ranged from 1.5 in an Asian cohort of 11,141 patients who were monitored through monthly interviews following a standardised protocol (Nolan, Goldberg & Buskin 1999) to 79.6 in a US cohort of 1,000 for which cases were defined biochemically (Byrd et al. 1979). In a US Public Health Service surveillance study involving 13,838 participants a more moderate incidence of 13.6 per 1,000 was reported (Kopanoff, Snider & Caras 1978). This 1978 study reported that increasing age was a predominant factor for higher risk of developing isoniazid-related hepatitis.

Other anti-TB drugs and regimens

Forget and Menzies (2006) reported incidence data for overall rates of side effects for patients on various anti-TB medications (Table 32). The authors report that populations, and

[13] Mortality rates were adjusted for compliance

[14] The authors calculated adjusted pooled incidence based on different definitions of hepatoxicity/hepatitis. The incidence was much lower (6.7/1000) in studies using clinical criteria to define hepatitis than in those using biochemical criteria alone

in particular, definition of AEs showed heterogeneity between studies (no statistical measure given). Also reported was that older age was associated with the development of hepatitis, and skin rashes were more common in patients who were female, older, HIV infected or from Asia. Patients with chronic renal failure tend to have a higher incidence of adverse effects to an anti-TB regimen, in particular neuropsychiatric events.

Interestingly, more patients discontinued treatment on isoniazid mono-therapy (7%) when compared with rifampicin (2%), pyrazinamide (5%), ethambutol (0.2–0.3%) and streptomycin (3%). Patients on isoniazid also had more-elevated simple transaminases than those on the other drugs, but experienced hepatitis less often (0.4%) than rifampicin (1%) or pyrazinamide (1.5%). In children on TB mono-therapies, AEs occurred less often than in adults and tended to result in less morbidity; however, a large proportion of this data came from studies of prophylactic treatment.

Table 32 Incidence of AEs for drugs and regimens for first-line and prophylactic TB treatment

Drug / regimen	Discontinuation of treatment (overall)	Hepatitis	Simple elevation of transaminases	Dermato-logical	Gastro-intestinal	Hyper-sensitivity	Neuro-logical*
INH	7%	0.4%	11%	1%	2%	0.1–17%	1–3%
RIF	2%	1%	3–9%	0.5–3%	1–8%	Yes	No
PZA	5%	1–5%	NA	2–5%	1%	NA	No
EMB	0.2–0.3%	Rare	No	Rare	No	No	0.2–0.3%
SM	3%	No	No	2%	No	Rare	Yes
RIF-PZA	8%	8%	6%	4%	6%	0.3%	0.1–3%
PZA-Q	67–88%	18%	4–88%	–	–	–	–
HR+	5%	3%	15%	3%	2%	2%	3–7%
HRZ+	4%	3%	22%	12%	12%	1–4%	7%
HRS+	6%	2%	3–51%	11%	16%	1–4%	14%

Median rate given for values representing ≥ 4 studies; range given for values representing ≤ 3 studies
[a] Includes vestibular toxicity and optic neuritis
NA = not available; INH = isoniazid; Q = quinolone; RIF = rifampicin; PZA = pyrazinamide; EMB = ethambutol; SM = streptomycin; HR+ = regimens containing INH, RIF and any other drug, but not PZA or SM; HRZ+ = regimens containing INH, RIF, PZA and any other drug, but not SM; HRS+ = regimens containing INH, RIF and SM and any other drug
Source: Forget and Menzies (2006)

Adverse reactions in children (0–18 years)

A review by Frydenberg and Graham (2009) was identified that discussed toxicity of first-line anti-TB regimens in children. On discussion of recommended doses for children, it was noted that the dosage level of isoniazid should depend on whether the child is a fast, intermediate or slow acetylator, as determined by the N-acetyltransferase 2 genotype. The slow acetylator genotype is associated with more AEs (Possuelo et al. 2008; Tostmann et al. 2008). Once again, the attribution of side effects to individual drugs was found to be difficult when the majority of treatments are multidrug regimens. Incidence of AEs attributed to

individual drugs are summarised in Table 33, stratified according to dose/regimen. In some cases the data from a number of studies using the same dose were combined. Reports of adverse reactions for mono-therapeutic use of rifampicin and pyrazinamide are scarce as they are most commonly used in multi-therapies. Furthermore, a large proportion of the included mono-therapy data in children comes from prophylactic use of anti-TB drugs.

The AEs reported in treatment trials of multidrug regimens in children are summarised in Table 34. For comparative studies the arm in which the AE occurred was not reported by Frydenberg and Graham (2009). The AEs of two Indian trials assessing more serious forms of TB in children are shown in Table 35. One of the trials compared a treatment regimen containing streptomycin for more severe TB with a regimen without streptomycin for children with less-severe disease. A second trial treated children with tuberculous meningitis.

Table 33 Incidence of adverse reactions to TB drugs in children

Drug Adverse reaction	Drug regimen	Frequency (N participants)	Studies providing evidence (K)
Isoniazide			
Hepatic reactions			
Severe hepatitis, hepatic failure	< 10 mg/kg	Occasional (NR)	2
Transaminase elevation, subclinical	Chemoprophylaxis (early months)	5–10%	5
	Chemoprophylaxis (9 months)	6%	1
	Chemoprophylaxis (3–4 months)	1.2%	1
Jaundice	Chemoprophylaxis 10 mg/kg	1 case (1,451)	4
Discontinuation of therapy due to hepatoxicity	Chemoprophylaxis 10 mg/kg	2 cases (1,451)	4
	Chemoprophylaxis 10–20 mg/kg	0 (> 6,000)	3
Neurologic reactions	Daily unspecified dose	13%	1
Vitamin B6 deficiency	Daily unspecified dose	0	1
Clinical pyridoxine deficiency	Daily 3–15 mg/kg	0	2
Rifampicin			
Hepatic reactions			
Hepatoxicity	Chemoprophylaxis	0 cases (25)	1
Discontinuation due to transaminase elevation	Chemoprophylaxis 10 mg/kg	1 case (157)	1
Ethambutol			
Ocular reactions			
Possible ocular toxicity	15–30 mg/kg	2 cases (3,811)	2

NR = not reported
Source: Frydenberg & Graham (2009)

Table 34 AEs reported in treatment trials for TB in children

Interventional regimen	Comparator	Adverse reaction	Cases (N)	Country (N participants)
Daily: RIF 10 mg/kg INH 10 mg/kg PZA 25 mg/kg	Twice weekly: RIF 15 mg/kg INH 15 mg/kg PZA 55 mg/kg	Significant side effect	0	South Africa (206)
Twice weekly: RIF 10–15 mg/kg INH 20–30 mg/kg PZA 50–60 mg/kg	Daily: RIF 10–15mg/kg INH 10–15 mg/kg PZA 20–30 mg/kg	Requiring modification of treatment Initial vomiting Mild joint pains	0 6 2	India (76)
Daily for 9 months: RIF 12 mg/kg INH 6 mg/kg	Intermittent for 6 months: RIF 12 mg/kg INH 15 mg/kg PZA 45 mg/kg	AEs	0	India (NR)
RIF 10–15 mg/kg INH 15 mg/kg	Various, all including: RIF 10–15 mg/kg INH 15 mg/kg	Transient hepatitis Vomiting Skin rash	4 1 1	India (83)
RIF 10–12 mg/kg INH 10–12 mg/kg PZA 30–35 mg/kg	NA	Serious adverse effects Temporary asymptomatic hyperuricaemia or transient elevation of transaminases	0 11	Greece (36)
Twice weekly for 6 months: RIF 10–20 mg/kg INH 20–40 mg/kg PZA 50–70 mg/kg	NA	Significant events interrupting treatment (vomiting, skin rash) Events not requiring interruption of treatment (vomiting or abdominal pain) Hepatitis, peripheral neuritis, joint pain	2 9 0	USA (175)
Daily for 2 months: RIF 10–15 mg/kg INH 10–20 mg/kg PZA 25–35 mg/kg Then twice weekly for 4 months: RIF 10–15 mg/kg INH 10–20 mg/kg	NA	Rash Jaundice Deafness Temporary allergy (desensitised by increasing doses)	12 2 1 4	Papua New Guinea (639)

INH = isoniazid; NA = not applicable, non-comparative study; PZA = pyrazinamide; RIF = rifampicin; SM = streptomycin
Source: Frydenberg & Graham (2009)

Table 35 AEs reported for treatment regimens for children with severe TB disease or TB meningitis

Interventional regimen	Comparator	Adverse reaction	Frequency—Intervention	Frequency—comparator	Country (N participants)
Daily in intensive phase of more severe disease: RIF, INH, PZA, EMB	Daily for less severe disease: RIF, INH, PZA	Hepatoxicity	2%	1%	India (323)
Children with TBM: INH 20 mg/kg	Children with TBM: INH 12 mg/kg	Jaundice	39%	12%	India (NR)

EMB = ethambutol; INH = isoniazid; PZA = pyrazinamide; RIF = rifampicin; TBM = tuberculous meningitis
Source: Frydenberg & Graham (2009)

Multidrug resistance after inappropriate TB treatment

An SR and meta-analysis by van der Werf et al. (2012) assessed the risk of acquiring MDR-TB after taking inappropriate TB medication. A literature search for studies down to cohort level that assessed TB regimens as a risk factor for multidrug resistance identified no relevant articles, so the authors widened the selection criteria. Studies were included in which treatment was provided to non-MDR patients if drug resistance and genotype of the isolated TB bacilli were documented before treatment started. The definitions used by the authors for an appropriate treatment regimen and for MDR-TB are tabulated in Table 36 and Table 37, respectively. Four cohort studies were identified and included in the SR.

Table 36 Appropriate treatment regimens for TB patients with strains that have certain drug-resistance patterns

Drug-resistance pattern	Appropriate treatment regimen
Pan susceptible	H-R and two other drugs in intensive phase and H-R in the continuation phase
H	H-R and two other drugs in intensive phase and H-R-E in the continuations phase [a]
Non-MDR-TB, R-susceptible	At least three drugs to which the strain is sensitive in the intensive and continuation phase [b]
Non-MDR-TB, R-resistant	At least four drugs to which the strain is sensitive in the intensive and continuation phase [b]

[a] Based on World Health Organization guidelines (WHO 2010)
[b] Based on World Health Organization guidelines (WHO 2008)
H = isoniazid; MDR = multidrug resistance; R = rifampicin; E = ethambutol; TB = tuberculosis
Source: van der Werf et al. (2012)

Table 37 Definitions of MDR-TB, acquired MDR-TB, recurrence, relapse and reinfection

Type of TB	Definition
MDR-TB	TB resistant to at least isoniazid and rifampicin
Acquired MDR-TB	A case with an initial strain susceptible to at least isoniazid or rifampicin that developed MDR-TB and has a genotyping pattern identical to the strain at diagnosis
Recurrence	A second episode of TB occurring after a first episode has been considered cured
Relapse	A second episode of TB occurring after a first episode has been considered cured with the same MTB strain as the first episode

MDR = multidrug resistance; MTB = *Mycobacterium tuberculosis*; TB = tuberculosis
Source: van der Werf et al. (2012)

The populations of the four included studies were diagnosed with TB proven either by culture, new AFB-positive sputum (two AFB-positive sputum smears or one AFB-positive smear and an abnormal chest radiograph consistent with TB), AFB-positive sputum (at least one sputum sample reading > 10 bacilli/100 fields by direct microscopy), or by both sputum AFB microscopy and culture. Results from two of the four cohort studies were used in a fixed-effects model meta-analysis as they included patients in exposed (i.e. those who received an inappropriate treatment) and unexposed (i.e. those who received appropriate treatment) groups. As the exposed and unexposed groups were drawn from the same population, the studies potentially minimised population selection bias, although baseline differences were not assessed. In one study all patients were considered to have undergone

inappropriate treatment as the continuation phase consisted of isoniazid and ethambutol and not isoniazid and rifampicin. In another cohort there were no events (i.e. the strain of TB at recurrence was different to the strain at initial infection), so neither of these latter two studies could be included in the meta-analysis. The applicability of the study cohorts to an Australian setting was low, as the studies were conducted either in areas where there is moderate to high prevalence of drug-resistant TB or in patients who had experienced a previous TB infection.

All studies performed DST before the start of treatment, as per the selection criteria of the review. The quality of the included studies was assessed to be moderate to high by van der Werf et al. (2012); a summary of the data is presented in Table 38.

Table 38 Patients who acquired MDR-TB following appropriate or inappropriate TB treatment

Study reference Country	Treatment appropriate based on DST	Non-MDR-TB patients treated (n)	Patients that failed treatment and acquired MDR-TB (n)	Patients with recurrence with acquired MDR-TB (n)
Sonnenberg et al. (2001) South Africa	Yes No	294 29	– –	0 0
Quy et al. (2003) Vietnam	Yes No	0 2,551	0 38	0 10
Cox et al. (2007) Uzbekistan	Yes No	240 74	1 9	– –
Matthys et al. (2009) Russian Federation	Yes No	127 62	0 5	– –

DST = drug susceptibility testing; MDR = multidrug resistance; TB = tuberculosis
Patients for whom it was unknown whether they acquired MDR-TB are not included as acquired MDR-TB
Source: van der Werf et al. (2012)

Patients included in the meta-analysis were shown to have a 27-fold increased risk of drug resistance if they received an inappropriate treatment regimen (RR=26.7, 95%CI 5.0, 141.7). When two patients for whom the strain of re-infection was not clear were excluded from the analysis, the risk was lower (RR=17.7, 95%CI 4.1, 77.6). Results are shown in Table 39. Heterogeneity was measured at 0.02 (df=1, p=0.88, I^2=0%) using a Chi-squared analysis.

Table 39 Meta-analysis of two studies showing the risk ratio of inappropriate treatment and risk of developing multidrug-resistant TB

Study reference Country	Inappropriate treatment N events (total)	Appropriate treatment N events (total)	Weight	Fixed RR IV (95%CI)
Cox et al. (2007)	9 (74)	1 (240)	66.4	29.19 (3.76, 226.62)
Matthys et al. (2009)	5 (62)	0 (127)	43.6	22.35 (1.26, 397.86)
Total (95%CI)	14 (136)	1 (367)	100.0	26.68 (5.02, 141.70)

RR = risk ratio
Source: van der Werf et al. (2012)

In summary, there are AEs and morbidity associated with anti-TB treatment. Patients who carry a resistant strain that can be identified by NAAT will possibly benefit from its early identification, followed by appropriate treatment. It should be noted that a patient receiving appropriate treatment for a resistant or non-resistant strain will still be at risk of adverse health events associated with that drug or regimen.

Data providing the evidence on AEs was non-comparative and came primarily from countries with high or medium incidence of TB, and therefore there is limited relevance in an Australian setting. Heterogeneity of reporting, dosing regimens and definitions of AEs (e.g. hepatitis) in studies makes it difficult to conduct a serious analysis of the data.

An important finding by van der Werf et al. (2012) was that patients were found to be at higher risk of developing MDR-TB if they received inappropriate compared with appropriate treatment (RR=26.7, 95%CI 5.0, 141.7). Appropriate treatment was as defined according to WHO treatment guidelines for MDR-TB (WHO 2008). Thus, from a public health perspective, earlier identification of drug resistant strains via NAAT could be beneficial in preventing inappropriate treatment and the further spread of MDR-TB.

The *Tuberculosis notifications in Australia, 2010 Annual Report*[15] found that 12% of culture isolates with available DST results showed resistance to at least one of the standard first-line anti-TB agents. Resistance to isoniazid (no rifampicin resistance) was shown in 4.7% of isolates. Resistance to at least isoniazid and rifampicin (MDR-TB by definition) was reported in 3.5% of cases but half of these were from the Papua New Guinea – Torres Strait Islands cross-border region and the remainder from recent immigrants. Thus, drug resistance is currently not a serious problem in Australia. Appropriate treatment regimens would enable physicians to continue to contain or even reduce the spread of drug-resistant TB cases in Australia.

Linked evidence of diagnostic effectiveness of NAAT in the diagnosis of NTM

Literature on NTM generally falls into four categories: MAC, which is associated with lung disease in immunocompromised patients; *M ulcerans*, which is associated with Buruli ulcer disease, a skin disorder endemic to certain regions of Africa but also identified in Australia (sometimes known as 'Daintree ulcer'); *M avium* spp. paratuberculosis, a strain found

[15] Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

predominately in animals, causing Johne's disease, which is implicated in Crohn's disease in humans; and the other less common mycobacteria, which form the fourth category.

Although there was a considerable body of literature about NTM, very little of it was relevant to the review. Many of the studies were case reports or outbreak investigations; most of the literature on Crohn's disease compared the presence of mycobacteria in people with and without Crohn's disease, rather than how it is diagnosed. Indeed, none of the literature on *M. avium* in Crohn's disease was eligible for inclusion in the review. No direct evidence was found comparing NAAT with culture. Thus, a linked evidence approach was used. However, the studies that met the inclusion criteria only reported on diagnostic accuracy. No studies reporting on the effect of a change in management resulting from the use of NAAT were identified.

Is it accurate?

Summary—What is the diagnostic accuracy of NAAT versus culture in the diagnosis of NTM?

Diagnostic accuracy meta-analyses were conducted for multiple comparisons and the results are summarised below.

Culture as the reference standard

It should be noted that culture is an imperfect reference standard. When compared with a clinical reference standard, only 46% (95%CI 27, 66) of those clinically diagnosed were culture-positive and only 31% (95%CI 4, 58) were AFB-positive.

- The median sensitivity of NTM-NAAT versus clinical diagnosis was 99% (range 98–99; k=2), indicating that many patients who are NTM-NAAT-positive and culture-negative would be diagnosed with clinical disease.

NAAT compared with culture

Meta-analysis was performed comparing two different NAATs with culture. NTM-NAAT detects NTMs in general by targeting either the 16S-23S rRNA sequence (k=3) or the gene encoding the 65-kDa heat shock protein (k=2); and MAC-NAAT specifically detects MAC strains (k=5).

The pooled sensitivity for NTM-NAAT compared with culture was 84% (95%CI 49, 97) and the specificity was 90% (95%CI 46, 99):

- 16% of patients had false-negative results and 10% of patients had false-positive results.

The pooled sensitivity for MAC-NAAT compared with culture was 59% (95%CI 35, 79) and the specificity was 100% (95%CI 99, 100):

- 41% of patients had false-negative results and no culture-negative patients had false-positive results.

The summary LR+ and LR– values for the ability of NAAT to correctly diagnose the presence or absence of NTM infections in patients when compared with culture suggest that:

- Patients with a positive MAC-NAAT result most likely had a culture-positive MAC infection, and patients with a negative result may or may not have had a culture-positive MAC infection.

Summary—What is the diagnostic accuracy of NAAT versus culture in the diagnosis of NTM?

- Patients with a positive NTM-NAAT were more likely to have an infection than not, and patients with a negative result were more likely to not have an NTM infection than to be falsely negative.

The SROC curve shows some threshold effect, suggesting that MAC-NAAT may be more sensitive and less specific than NTM-NAAT when compared with culture:

- The AUC indicated that both NTM- and MAC-NAAT perform well in predicting culture positivity.

Overall, NAAT appears to be able to identify a larger proportion of patients with an NTM infection than either AFB microscopy or culture. However, only NTM-NAAT may be of any use in identifying those patients who do not have an NTM infection. Furthermore, these results should be viewed with caution due to the small number of studies included and the wide 95%CIs for many of the analyses.

Studies were included to assess the accuracy of NAAT according to criteria outlined in Box 5.

Box 5 PICO criteria for direct evidence in patients with tissue biopsy consistent with NTM infection

Population	Patients with tissue biopsy consistent with NTM infection
Intervention	Culture plus NAAT for the detection of non TB-mycobacteria (e.g. *Mycobacterium avium, M. kansasii, M. gordonae*, or *M. intracellulare*)
Comparators	Culture plus other tests, e.g. lung biopsy or skin biopsy if possible
Outcomes	Safety—adverse events from testing procedures and subsequent treatments Direct effectiveness—time to symptom resolution, quality of life
Study design	Randomised trials, cohort studies, case series or systematic reviews of these study designs
Search period	1990 – May 2014 or inception of the database if later than 1990
Language	Studies in languages other than English will only be translated if they represent a higher level of evidence than that available in the English language evidence-base

Note: The patient population was expanded to include all patients suspected of having an NTM infection.

Due to the paucity of evidence, variations from the PICO were necessary. The patient population for eligible studies was expanded to include any patients suspected of having an NTM infection. Although the protocol for this review stated that the reference standard should be AFB microscopy and culture, as so little evidence was found, studies with clinical reference standards (i.e. various combinations of clinical assessment and pathology results, but also response to treatment) were also included. Twelve studies conducted between 1997 and 2005 were identified that reported on the diagnostic accuracy of NAAT for the detection of NTM infections. The study profiles and the quality appraisal are summarised in Table 101 (Appendix F) and the extracted 2x2 data are presented in Table 91 and Table 92 (Appendix C). An overall summary of the body of evidence is presented in Table 40.

Table 40 Body of evidence matrix for studies reporting on the accuracy of NAAT in diagnosing NTM infections

Component	A Excellent	B Good	C Satisfactory	D Poor
Evidence-base [a]			One or two level III studies with a low risk of bias, or level I or II studies with a moderate risk of bias	

Component	A Excellent	B Good	C Satisfactory	D Poor
Consistency [b]		Most studies consistent and inconsistency may be explained		
Clinical impact				Slight or restricted
Generalisability			Population(s) studied in body of evidence differ to target population for guideline but it is clinically sensible to apply this evidence to target population	
Applicability			Probably applicable to Australian healthcare context with some caveats	

[a] Level of evidence determined from the NHMRC evidence hierarchy (see Table 13).
Source: Adapted from NHMRC (2009)

Ten studies reported the diagnostic accuracy of NAAT compared with culture, but only 6 of these studies also compared AFB microscopy with culture (Table 101). Two of the studies also reported the diagnostic accuracy of NAAT and culture compared with a clinical reference standard. An additional two studies reported on the diagnostic accuracy of AFB, NAAT and culture compared with a clinical reference standard only (Table 101). Culture methods included standard diagnostic laboratory procedures, including L-J or Ogawa solid media and/or liquid BACTEC media. Four studies included respiratory specimens (3 of which included sputum specimens and 1 also included extrapulmonary specimens), 4 used blood and bone marrow specimens (all from HIV-positive patients) and 4 used tissue biopsy specimens (3 used archived formalin-fixed paraffin-embedded specimens).

The NAAT used in these studies could be separated into three distinct categories. Five studies used NAAT to detect NTMs in general (NTM-NAAT) by targeting either the 16S–23S rRNA sequence (k=3) or the gene encoding the 65-kDa heat shock protein (k=2). Six studies used NAAT to specifically detect MAC strains (MAC-NAAT), which included all 4 studies involving HIV-positive patients (1 of which only used a clinical reference standard). One study used NAAT to detect *M. ulcerans* in patients suspected of having Buruli ulcer by targeting IS*2404*, but only compared NAAT with a clinical reference standard (Table 101). However, many of these studies also identified patients (specimens) with MTB infections (Table 91 and Table 92). As MTB infections are much more common than NTM and would therefore affect the accuracy of NAAT in detecting NTM, MTB culture-positive specimens were excluded from the analysis wherever possible. MTB-positive results could not be excluded from the analysis for 2 studies that identified 6/46 (Bogner et al. 1997) and 2/36 (Mahaisavariya et al. 2005) positive cultures as MTB, and the study by Frevel et al. (1999) did not report the number of MTB-positive cultures included in the analysis.

The prevalence of patients with culture-positive NTM infections varied between 4% and 67%, with 5 studies reporting NTM-positive cultures in less than 10% of the tested specimens. Only 2 studies reported a prevalence rate greater than 30%; these were 42% in the study investigating the presence of *M. ulcerans* in biopsy specimens from a suspected Buruli ulcer (Phillips et al. 2005) and 67% in a study investigating the presence of MAC in blood and bone marrow aspirates from AIDS patients who were suspected of having disseminated mycobacterial infections (Gamboa et al. 1997). The reason for this high rate of culture-positivity when compared with other studies looking at disseminated mycobacterial infections could not be determined. Studies using NTM-NAATs reported a mean prevalence of NTM-positive cultures of 13% (range 4–30) compared with 25% (range 9–67) for those using MAC-NAATs. As expected, the prevalence of culture-positive NTM was higher in AFB-positive specimens (36%) compared with AFB-negative specimens (21%; Table 41).

Table 41 Prevalence of NTM culture-positive specimens in the included studies

Specimen type	Number of studies	Prevalence of culture-positive NTM
All specimens across all studies	7	14% [range 4–30]
NTM-NAAT vs culture studies	5	13% [range 4–27]
MAC-NAAT vs culture studies	5	25% [range 9–67]
AFB-positive specimens	3	36% [range 11–60]
AFB-negative specimens	5	18% [range 4–67]

AFB = acid-fast bacilli; NTM = non-tuberculous mycobacteria; NTM-NAAT = NAAT designed to detect all NTMs; MAC-NAAT = NAAT designed to detect *M. avian* complex; NAAT = nucleic acid amplification testing

The sensitivity and specificity of AFB, NTM-NAAT, MAC-NAAT and culture compared with either culture or a clinical reference standard for the individual studies are shown in Figure 49 and Figure 50 (Appendix D), and the pooled values for various subgroups are shown in Figure 30.

When NTM-NAAT was compared with MAC-NAAT using culture as the reference standard, NTM-NAAT was more sensitive than MAC-NAAT (84%; 95%CI 49, 97 versus 59%; 95%CI 35, 79), but this difference did not reach statistical significance due to the wide CIs (Figure 30). The difference in sensitivity between MAC-NAAT and NTM-NAAT may be due to the restricted mycobacterial species detectable using MAC-NAATs. Two studies that used commercial MAC-NAATs (Gamboa et al. 1997; Ninet et al. 1997) identified 13% (5/38) and 9% (6/68) culture-positive specimens, respectively, that grew NTMs not detectable by MAC-NAAT that could have been detected by NTM-NAAT. These specimens were treated as falsely negative in the analysis presented in this report. Conversely, 2 other studies included specimens with cultures positive for other NTMs as culture-negative results in the data presented (Bogner et al. 1997; Tran et al. 2014). Thus, 4% (18/494) and 7% (25/361) of

culture-negative specimens, respectively, were actually NTM culture-positive, thus overestimating the sensitivity of MAC-NAAT in the detection of patients with NTM infections in these 2 studies. The study by Matsumoto et al. (1998) did not report the presence of any other NTMS.

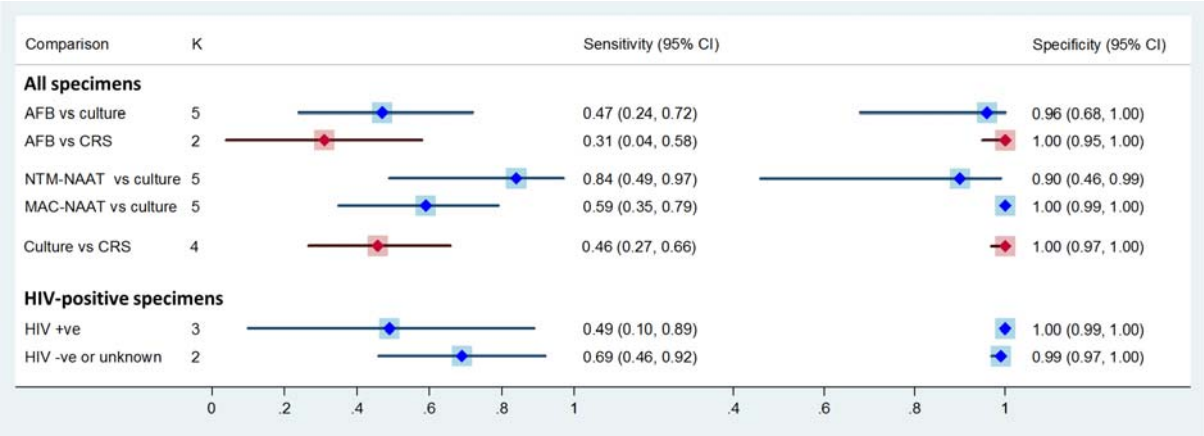

Figure 30 Forest plot showing the pooled sensitivity and specificity values for AFB and NAAT compared with culture or a clinical reference standard in diagnosing NTM infections in various types of specimens

Comparisons using culture as the reference standard are shown in blue and those using a clinical reference standard in red. When there were 4 or more studies, pooled values were obtained using the 'midas' command in Stata; when there were less than 4 studies the pooled values were estimated using the 'metan' command.

AFB = acid-fast bacilli; CRS = clinical reference standard; NTM-NAAT = NAAT designed to detect all non-tuberculous mycobacteria; MAC-NAAT = NAAT designed to detect *M. avian* complex; NAAT = nucleic acid amplification testing

AFB microscopy was not very useful in identifying patients who did not have NTM infections. The pooled sensitivity for AFB microscopy versus culture was 47% (95%CI 24, 72), indicating that 53% of patients with a positive culture would have a false-negative result. When compared with a clinical diagnosis based on symptoms, histopathology and culture results, 77% of those diagnosed had a false-negative AFB result (pooled sensitivity 31%; 95%CI 4, 58). However, the pooled specificity was 97–100%, indicating that few patients would have a false-positive AFB result (Figure 30). The LR scattergram in Figure 31A shows that the summary LR+ and LR– estimates for AFB microscopy are in the upper right quadrant, indicating that patients with a positive AFB microscopy result are indeed very likely to have an NTM infection detectable by culture. However, a negative AFB result does not exclude the possibility of having an NTM infection and has no diagnostic value.

When comparing AFB microscopy with NTM-NAAT using culture as the reference standard, the pooled estimates suggested that NTM-NAAT was more sensitive (47% versus 84%; Figure 30), but this was not statistically significant as the CIs overlapped. There was a smaller difference in the pooled sensitivity for AFB microscopy compared with MAC-NAAT, with the CIs almost completely overlapping (47% versus 59%; Figure 30). Nevertheless, fewer patients would receive a false-negative result with NAAT compared with AFB

microscopy, with up to 23% of NTM culture-positive patients being AFB-negative and NTM-NAAT-positive.

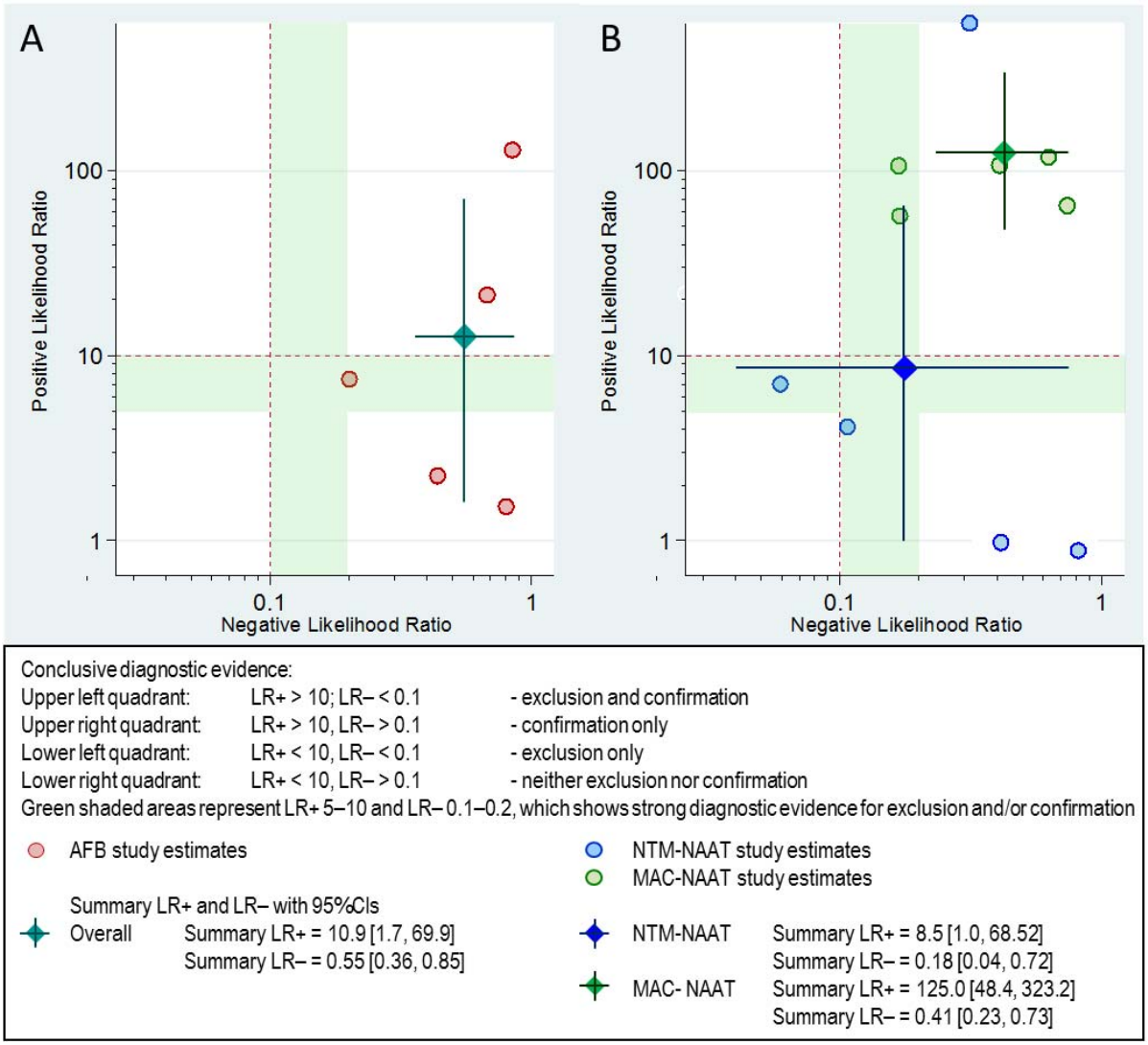

Figure 31 LR scattergram for diagnosis of NTM infection by AFB microscopy (A) and NAAT (B) compared with culture

AFB = acid-fast bacilli; NTM-NAAT = NAAT designed to detect all non-tuberculous mycobacteria; MAC-NAAT = NAAT designed to detect *M. avian* complex; NAAT = nucleic acid amplification testing

NTM-NAAT was less specific than MAC-NAAT using culture as the reference standard (90%; 95%CI 46, 99 versus 100%; 95%CI 99, 100), but did not differ significantly to that for AFB microscopy (96%; 95%CI 68, 100). It should be noted that culture is an imperfect reference standard. When compared with a clinical reference standard, only 46% (95%CI 27, 66) of those clinically diagnosed were culture-positive, and only 31% (95%CI 4, 58) were AFB-positive. The median sensitivity for NTM-NAAT (99%, range 98–100, k=2; Figure 49) was higher than for culture and the specificity ranged from 87–100%. This suggested that most patients who were NTM-NAAT-positive and culture-negative probably had clinical disease.

In Figure 31, the LR scattergram showed that the summary LR+ and LR– values were in the top right quadrant, suggesting that a patient with a positive MAC-NAAT most likely had a MAC infection detectable by culture. However, a negative MAC-NAAT result does not eliminate the possibility of being culture-positive. On the other hand, the summary LR+ and LR– values for NTM-NAAT were within the green shaded areas, indicating that a patient with a positive NTM-NAAT is more likely to be culture-positive than not, and that a negative NTM-NAAT may be suggestive of not having a culture-positive infection. Thus, NAAT performed similarly to AFB microscopy in the ability to confirm the presence of culture-positive NTM infections, but NTM-NAAT was more likely to correctly predict the absence of disease.

The SROC curve, which depicts the relative trade-off between true-positive and false-positive results, shows a trend indicating that there may be a threshold effect between MAC and NTM-NAATs (Figure 32). The AUCs for NTM-NAAT (0.92; 95%CI 0.90, 0.94) and MAC-NAAT (1.00; 95%CI 0.98, 1.00) indicate that the NAATs perform well in predicting culture positivity (AUC > 0.9), whereas AFB microscopy (0.76; 95%CI 0.72, 0.79) performs only moderately (AUC 0.7–0.9). There was also a threshold effect between NTM-NAAT and MAC-NAAT indicating that NTM-NAAT is more sensitive and less specific.

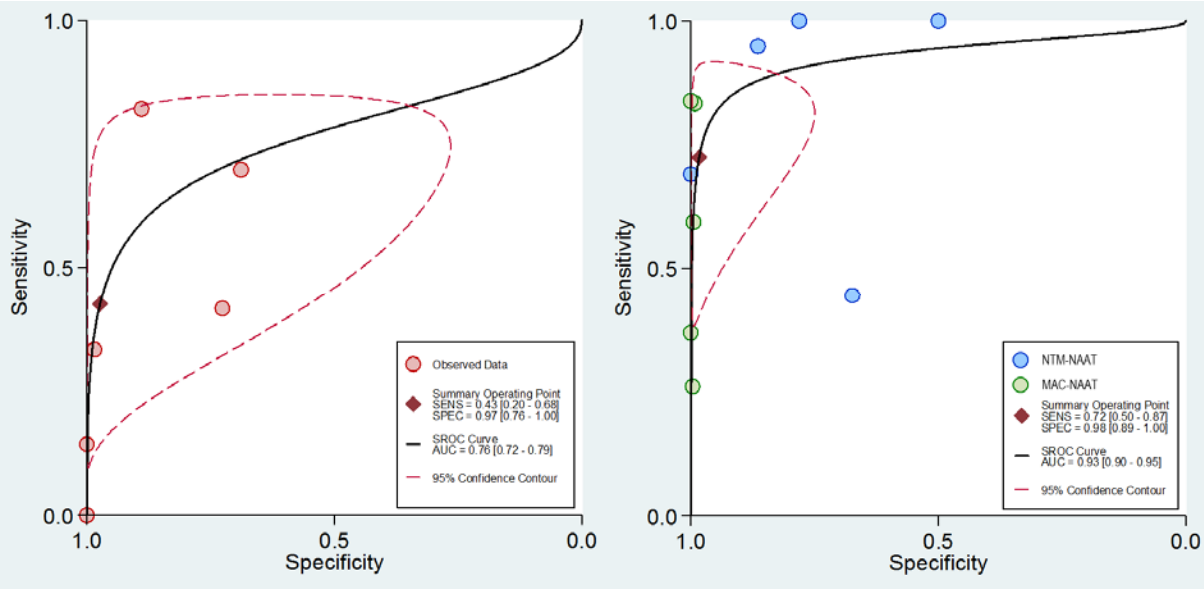

Figure 32 SROC curve for all studies investigating the sensitivity and specificity of AFB and NAAT versus culture in the diagnosis of NTM

AUC = area under curve; SROC = summary receiver–operator characteristic; NTM-NAAT = NAAT designed to detect all non-tuberculous mycobacteria; MAC-NAAT = NAAT designed to detect *M. avian* complex; NAAT = nucleic acid amplification testing

Although the pooled sensitivity of the 3 MAC-NAAT studies that included HIV-positive patients was only 49% compared with 69% for the other 2 MAC-NAAT studies, this

difference was not significant as the wide CIs almost completely overlapped (Figure 50 in Appendix D). Thus, there is no obvious difference in test performance in specimens from HIV-positive patients compared with those that are HIV-negative.

No conclusions could be reached about the accuracy of NAAT in the diagnosis of NTM in AFB-positive or -negative specimens. Not surprisingly, many specimens included in the analysis were AFB-negative (mean 16%, range 0-39), largely due to the paucibacillary nature of many specimen types tested for NTM infections. As a result, only 3 studies provided any data on AFB-positive specimens, 2 of which used NTM-NAAT and 1 MAC-NAAT. When these studies were compared with the 6 studies (3 NTM-NAAT and 3 MAC-NAAT) that provided data on AFB-negative specimens, the variability between studies was so great that no conclusions could be reached (Figure 50 in Appendix D).

Together, these results suggest that NAAT may be a better diagnostic test for diagnosing NTM infections than either AFB microscopy or culture. However, the results should be viewed with caution due to the small number of studies included and the wide 95%CIs for many of the analyses.

Other relevant considerations

TB in the Australian Indigenous population

High incidence of TB among Indigenous Australians

Although rates of TB in Australia are low, the absolute numbers of TB cases increased by 33% between 1998 and 2008, and specific subgroups such as indigenous Australians and immigrants have much higher rates than other Australians. The *Tuberculosis notifications in Australia, 2010 Annual Report*[16] found that the incidence of TB in the Australian-born Indigenous population was 11 times higher than in the Australian-born non-Indigenous population (7.5 versus 0.7 per 100,000 people).

The *Strategic Plan for Control of Tuberculosis in Australia: 2011–2015*[17] reported that rates of TB infection increase with age and transmission of TB to infants and children still occurs. Indigenous Australians also have higher rates of hospitalisation and mortality from TB than non-Indigenous Australians. Testing has shown that clustering of cases in households, and remote and town-camp communities occurs.

Addressing the problem

Strategies and policies such as *Closing the Gap*[18] and *The Strategic Plan for Control of Tuberculosis in Australia: 2011–2015* are aimed at addressing the high Indigenous TB rates. Key priorities and actions for TB control that impact on Indigenous Australians include:

- reducing the disparities in TB rates among population sub-groups within Australia
- minimising the development of drug resistance within Australia
- ensuring the continued provision of safe, timely laboratory diagnosis of TB
- developing a strategy for awareness campaigns for primary care and organisations representing high-risk groups
- developing a national strategy for long-term assured supply of quality TB diagnostics and medications.

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[16] Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

[17] Available from URL: http://search.informit.com.au/documentSummary;dn=967413476493835;res=IELHEA (accessed 13 June 2014)

[18] Available from URL: http://www.healthinfonet.ecu.edu.au/closing-the-gap/key-facts/what-is-closing-the-gap (accessed 3 November 2014)

Skilled clinical and laboratory staff and universal access to rapid and reliable diagnosis and treatment for TB are critical for the success of these measures[19]. There are five state Mycobacterium Reference Laboratories in Australia, which provide basic TB diagnostic services (AFB microscopy and culture) as well as NAAT, DST, rapid molecular detection of drug resistance, and molecular epidemiological typing. These laboratories also provide specialised diagnostic services for the detection and characterisation of clinically significant NTM infections.

In providing these services, laboratories face increasing challenges such as the rising costs of providing a range of NAATs and compliance with progressively more stringent biosafety standards. Thus, the laboratories require the continued support of federal and state governments to remain an integral part of the nation's TB control program. Currently, the cost of NAAT is mostly covered by state funding to the laboratories, but the availability of reimbursement for NAAT on the MBS would aid laboratories in maintaining the current high standard of the services provided. MBS reimbursement would also enable other public and private laboratories to offer NAAT in cooperation with the reference laboratories, which would provide training of laboratory personnel in mycobacterial diagnostics in both the public and private sectors. The broader availability of NAAT may result in more-rapid diagnosis and treatment of TB, leading to a further reduction in the spread of TB among close contacts in the community. This would benefit both the Indigenous and the immigrant populations.

Point-of-care NAAT for the detection of MTB and rifampicin resistance

Indigenous Australians who live in remote communities face specific challenges in being able to access healthcare initiatives such as TB control programs. Rapid diagnosis and treatment is essential to contain the spread of TB in these communities, especially to children and infants. Thus, point-of-care testing with same-day results offers easier access to diagnosis and more-rapid treatment initiation for people living in these isolated communities.

Xpert is the first fully automated NAAT developed for point-of-care diagnosis of MTB and rifampicin-resistant MTB, and was endorsed by the WHO in December 2010 (WHO 2014). WHO stated that 'Xpert testing should not be placed solely in centralized reference laboratories since patients gain the greatest benefit from the test when it is placed as close

[19] *The Strategic Plan for Control of Tuberculosis in Australia: 2011–2015*. Available from URL: http://search.informit.com.au/documentSummary;dn=967413476493835;res=IELHEA (accessed 13 June 2014)

as possible to the point of care'. However, WHO also noted that certain conditions and infrastructure need to be available to ensure its efficient use. These include a stable and continuous electrical supply, an ambient temperature of 15–30 °C in the testing room, trained staff to perform the test, and biosafety precautions similar to those needed for direct AFB microscopy.

Three studies that met the inclusion criteria and looked at the use of Xpert in a point-of-care setting were included in this report. Only 1 of these studies provided any diagnostic accuracy data, reporting on the concordance between Xpert conducted by a nurse and by a trained laboratory technician. All 3 studies reported on differences in time to treatment initiation, with 2 studies also reporting health-related treatment outcomes.

A randomised, parallel-group, multicentre trial conducted by Theron et al. (2014) randomised adults with symptoms suggestive of active TB from five primary healthcare facilities in South Africa, Zimbabwe, Zambia, and Tanzania to nurse-performed Xpert NAAT or sputum AFB microscopy at the clinic. In this study nurse-administered Xpert had substantial agreement with that done by a laboratory technician on a paired sputum specimen (κ=0·69; 95%CI 0·64, 0·74), and had a similar sensitivity and proportion of unusable results. The authors reported that nurse-administered Xpert detected 154 (83%) of 185 culture-positive patients, 112 of whom started treatment on the same day. However, as AFB microscopy was also done on site, the delay in treatment initiation in 91 (50%) of 182 culture-positive patients was only 1 day (IQR 0–4). Thus, it was not surprising that there were no significant differences in morbidity and mortality at either 2 months or 6 months follow-up between the two groups. Nevertheless, nurse-administered Xpert detected a larger proportion of culture-positive cases than AFB microscopy (83% versus 50%). Thus, more patients would start treatment immediately after Xpert than after AFB microscopy.

A cohort study by Van Rie et al. (2013a) reported on the use of Xpert at a large primary care clinic in South Africa between April and October 2010. On presentation, two sputum samples were collected for AFB microscopy (and NAAT if the patient consented to participate in the study), and the patient was given a 5-day course of antibiotics if clinically indicated and asked to return within 5–7 days. Individuals returned to the clinic for their results after a median of 8 days (IQR 6–22). A third sputum sample was then collected and sent to a central laboratory for fluorescent AFB microscopy and liquid culture. The authors reported that patients who were Xpert-positive were started on anti-TB treatment on the same day as collection of the third sputum specimen in 15/16 cases, compared with a median delay of 13 days (IQR 7–27) for 38 patients diagnosed by chest X-ray and 34 days for

1 patient diagnosed by culture. Three patients were identified as AFB-positive and Xpert-negative (two were also culture-negative); however, none of them started treatment due to unsuccessful tracing.

A third study by Hanrahan et al. (2013) was conducted between July and September 2011 at the same South African primary care clinic as the study by Van Rie et al. (2013a). In this study 96% (48/50) of Xpert-positive patients were started on treatment on the same day as presenting with symptoms (IQR 0–0), compared with a treatment delay of 14 days (IQR 7–29) for 18 Xpert-negative patients who were diagnosed by chest X-ray, 144 days (IQR 28–180) for 14 patients diagnosed by culture and 14 days (IQR 5–35) for those diagnosed empirically according to symptoms. However, at 6 months, treatment outcomes did not differ significantly between patients who were initially Xpert-positive or -negative (p=0.46). Among the 48 Xpert-positive cases started on treatment, 48% had a successful treatment outcome (i.e. 6-month treatment completion or cure) and 2% died. Among the 58 Xpert-negative patients started on treatment, 64% completed the treatment or were cured, and 2% died.

Thus, Xpert could be suitable for use in small regional hospitals and clinics in rural areas of Australia if suitable training of personnel was available. This would reduce the time between specimen collection and availability of test results for people living in remote communities, and may result in quicker treatment initiation. In addition, the early knowledge of rifampicin resistance may influence treatment decisions, ensuring that appropriate anti-TB drugs are given immediately. The linked evidence on patient outcomes due to a change in management indicated that there does not appear to be any advantage for patient health-related outcomes (e.g. cure) with early versus delayed treatment. However, early appropriate drug treatment reduced both the spread of TB to close contacts and the likelihood of developing drug resistance. Both of these are important public health outcomes essential for the control of TB in Australia.

What are the economic considerations?

Economic evaluation

Overview

A cost–utility analysis is presented to assess the cost-effectiveness of adding NAAT to AFB smear microscopy, and culture and sensitivity (C&S) testing in a population with clinical signs and symptoms of active TB. This is consistent with previously published economic evaluations of NAAT identified in the international literature. The economic model takes the form of a decision tree analysis, incorporating estimates of TB prevalence in the tested population, and AFB microscopy ± NAAT accuracy. The time horizon of the model is 20 months, chosen to capture all related costs and health outcomes in patients treated for TB ± MDR. Costs captured in the economic modelling include those of treatment, treating AEs, monitoring/management, hospitalisation and secondary transmissions. Outcomes were measured in quality-adjusted life years (QALYs), which were adjusted to capture disutility associated with treatment, and a further utility penalty was applied to account for decreased outcomes associated with active TB transmissions.

Four scenarios were considered in the economic analyses, based on the involvement of clinical judgment in initial treatment decisions (i.e. in determining the pre-test probability of TB). Various sensitivity analyses were also undertaken. The ICER of NAAT in the scenario thought to best reflect current practice (the 'TB mixed scenario') is $90,728/QALY. The incremental costs were observed to be driven largely by the cost of NAAT ($130). The ICER is most sensitive to decreases in the prevalence of TB in the tested population and in the specificity of NAAT, particularly in those with AFB-negative results and for rifampicin resistance.

Population and setting for the economic evaluation

The PASC protocol listed three populations with suspected active mycobacterial infections that would be considered potentially eligible for MBS-funded NAAT. These are patients:

- with clinical signs and symptoms of active TB whose specimen is able to have AFB microscopy and C&S testing
- with clinical signs and symptoms of active TB whose specimen is not able to have AFB microscopy but who have C&S testing
- suspected of having an NTM infection who are able to have C&S testing.

NAAT is proposed to be undertaken as an additional test to existing test procedures in all these populations.

For patients suspected of TB the protocol indicates that initial treatment decisions are based on the clinical suspicion (pre-test probability) of TB, based on clinical judgement of the background epidemiology of the patient, presenting symptoms and imaging features. If TB is clinically suspected, patients are currently initiated on treatment irrespective of the AFB result. However, if the clinical suspicion of TB is deemed low, the decision to initiate treatment is based on AFB, if able to be performed. This is consistent with the current clinical management algorithm presented in Figure 3.

The introduction of NAAT is not expected to alter treatment initiation decisions in patients with a strong clinical suspicion of TB. However, as NAAT has the ability to identify mutations associated with rifampicin resistance, an appropriate MDR-TB treatment regimen may be initiated sooner in those identified with rifampicin resistance. In patients with a low clinical suspicion of TB, the PASC protocol considers that treatment decisions would be based on the NAAT result if AFB and NAAT are discordant. This is consistent with the proposed clinical management algorithm presented in Figure 3.

There is inadequate evidence available to demonstrate the effectiveness of NAAT in the second and third populations, and so economic evaluations for these populations would be inappropriate. Any health outcome difference incorporated into the model would not be evidence-based and therefore could only be speculative. Subsequently, a calculation of cost-effectiveness would be inappropriate as it would generate results that do not have an evidentiary basis. Any ICER would be subject to an unacceptable level of uncertainty and could be potentially misleading. However, a costing assessment has been undertaken of the financial implications for the MBS and Australian governments should the proposed listings for the second and third populations be accepted (see 'Financial implications')

The PASC protocol indicated that, due to possible differences in the accuracy of NAAT in patients with and without HIV, separate analyses should be presented (with the proposed structure of the decision analytic also considering HIV subgroups). However, as the clinical assessment found little difference in the accuracy between these populations (see 'Comparison of AFB microscopy and NAAT, using culture as a reference standard in HIV-positive and HIV-negative patients'), and no evidence for change in management was identified, this subgroup has not been modelled separately. As all patients suspected of TB who are known to have an HIV infection would be considered to have a high clinical suspicion of TB, treatment is likely to be initiated on the basis of this suspicion. In this

respect, the modelled scenario that best represents this population is that in which all patients are considered to have a high clinical suspicion of TB (see 'Modelled economic evaluation').

Structure and rationale of the economic evaluation

Economic literature review

A literature search was conducted to identify published economic evaluations of NAAT for active TB infections (in those who can have an AFB) and to inform the structure of and inputs to the economic model (see Appendix H).

Five studies were identified that investigated the cost-effectiveness of NAAT in low-prevalence populations, as these are the most relevant to the Australian population (Table 42) (Choi et al. 2013; Dowdy et al. 2003; Hughes et al. 2012; Millman et al. 2013; Rajalahti et al. 2004).

Table 42 Economic evaluations identified that investigate NAAT for active TB in low-prevalence countries

Study	Setting	Results
Millman et al. (2013)	Decision tree analysis of adult inpatients in US hospital setting who have presumed TB and are in isolation until results of diagnostic tests (AFB compared with NAAT) become available. Differences in health outcomes were not anticipated, and so net costs were determined, which considered savings associated with the reduction in unnecessary hospitalisations and isolations. The cost implications of FPs and FNs were additionally considered as a cost penalty.	NAAT was associated with cost savings due to reduced hospital isolation and reduced overall length of stay.
Choi et al. (2013)	Decision tree cost–utility analysis of individuals with suspected pulmonary TB in the USA. A single-year time horizon was used for mapping the decision analytic, after which extrapolation extended the time horizon to the life expectancy of the patients. Models are for HIV-negative and HIV-positive patients (considering different epidemiological and accuracy estimates, but same utility weights), and include outcomes of resistance mutation testing. Costs included lab testing, hospitalisation, isolation and treatment. Implications for FPs were considered. Multiple testing algorithms were modelled. Algorithm 1 (no molecular testing) is relevant to comparator, with algorithms 3 and 5 relevant to proposed NAAT in Australia. Treatment may be initiated in AFB-negative, NAAT-negative if clinical suspicion is high (i.e. clinical diagnosis) (any algorithm)	Testing without NAAT was dominated by the strategies that included NAAT.

Study	Setting	Results
Hughes et al. (2012)	Decision tree cost–utility analysis of NAAT for people with a clinical suspicion of TB in the UK setting. Time horizon chosen of 1 year. Model incorporates resistance testing and outcomes in FP and FNs. Costs include testing, treatment and follow-up outpatient consultations; isolation costs were not considered. The model did identify the number of people infected by unidentified TB, but attributed neither their costs nor outcomes into the results. Strategies relevant to this model include #3: AFB and culture every time (for the comparator); and #11: AFB, NAAT and culture every time (for NAAT).	Strategy #11 was unlikely to be cost-effective compared with #3 (ICER £64,723). If secondary infections were incorporated fully into the model, the authors conclude that it could be conceivable that #11 would be optimal, as it was associated with the fewest secondary infections.
Rajalahti et al. (2004)	Decision tree cost-effectiveness analysis of AFB and culture ± NAAT in patients with a clinical suspicion of TB in the Finnish setting. Effectiveness was measured in terms of correct treatment and isolation decisions. Costs included isolation, treatment, lab tests and inpatient/outpatient visits. Decision tree parameters populated based on observed data.	NAAT was associated with additional costs when applied in all patients, but cost savings were only in AFB-positive patients.
Dowdy et al. (2003)	Decision tree cost-effectiveness analysis of NAAT in AFB-positive patients in the US setting. Effectiveness was measured in terms of 'early exclusion of TB'. Costs included testing, isolation and treatment. Unclear if all patients were subject to C&S testing.	NAAT was not considered cost-effective, as costs did not offset those of isolation and treatment averted.

AFB = acid-fast bacilli; C&S = culture and sensitivity; FN = false negative; FP = false positive; HIV = human immunodeficiency virus; NAAT = nucleic acid amplification testing; TB = tuberculosis

All 5 studies were generally consistent in structure (decision tree) and time horizon (up to 1 year). Three of the studies considered the implications of false-positive and false-negative results, and most considered the cost of hospital isolation. However, the outcomes of the models varied; 2 studies (Choi et al. 2013; Hughes et al. 2012) measured outcomes in terms of cost per QALY, whereas the other 3 investigated cost per correct treatment or isolation decision, or just costs as no change in outcomes were anticipated. The studies additionally varied in their results, with NAAT considered cost-effective in 3 studies and not cost-effective in 2.

None of the identified studies were conducted in the Australian setting. In Australia treatment initiation decisions take the clinical suspicion of TB into consideration. However, clinical suspicion was not considered in any of the studies identified, and therefore the applicability of the identified economic evaluations to the Australian context is uncertain. A modelled economic evaluation will be presented to determine the cost-effectiveness of NAAT (as an add-on test) in the population who can currently have an AFB.

Modelled economic evaluation

The structure of the economic model has been adapted from the cost–utility analyses identified in the literature search (Choi et al. 2013; Hughes et al. 2012) to suit the local

context. As clinical management in Australia differs depending on the clinical suspicion of TB, the model will be separated into patients with:

- a high clinical suspicion of TB (where treatment is initiated based on clinical suspicion)
- a low clinical suspicion of TB (where treatment decisions are initiated or delayed based on AFB ± NAAT results).

The benefit of NAAT in patients with high clinical suspicion of TB is to identify resistance mutations and initiate appropriate treatment for MDR earlier. In addition to earlier MDR treatment initiation, patients with low clinical suspicion of TB have additional benefits: NAAT may differentiate between TB and NTM infections (who would have been previously treated on the basis of the AFB results alone), and may reduce the delay in treating those with true TB who returned a negative AFB result (who would not have been treated without the availability of NAAT).

The model will take the form of a cost–utility analysis as this enables an assessment of NAAT in the context of the proposed benefits described above, in addition to quantifying the cost and outcome implications of false-positive and false-negative results (for the AFB microscopy ± NAAT alternatives).

A time horizon of 20 months was chosen, although this is longer than previously published cost–utility analyses, to capture all costs and outcomes associated with treatment for all patients, as treatment beyond 1 year is standard in patients with MDR-TB.

A summary of the structure of the economic model is presented in Table 43.

Table 43 Summary of the economic evaluation

Time horizon	20 months
Outcomes	QALYs
Costs	Australian dollars, 2014 prices
Methods used to generate results	Decision tree analysis
Discount rate	5% costs and outcomes accrued beyond 1 year
Software packages used	Microsoft Excel

QALY = quality-adjusted life-year

The structure of the decision tree is presented in Figure 33 (AFB model arm) and Figure 34 (AFB plus NAAT model arm).

Currently, patients with true TB are likely to be mixed across the populations that have a high or low clinical suspicion of TB. The prevalence of TB in each of these patient groups is likely to vary, as it would be expected that those with a high clinical suspicion of TB would

have a higher prevalence than those with a low clinical suspicion of TB. These assumptions are used to inform the base-case scenario ('TB mixed scenario'). However, given the influence of clinical judgement on the treatment management pathways, and the uncertainties associated with estimating the relative mix of patients across these groups (see 'Prevalence of TB'), the influence of clinical judgment on the cost-effectiveness of NAAT will be explored through the addition of the following scenarios:

- TB low suspicion scenario: all patients (including all with true TB) are treated as though they have a low clinical suspicion of TB (i.e. clinical judgment is not used as a basis to initiate treatment)
- Perfect clinical judgment scenario: all patients with true TB are treated as though they have a high clinical suspicion of TB (i.e. clinical judgement is used as a basis to initiate treatment, and it is assumed that this has 100% sensitivity and specificity in identifying TB), and all patients without TB are treated as though they have a low clinical suspicion of TB (i.e. treatment initiation decisions are based on results of AFB ± NAAT)
- TB high suspicion scenario: all patients are treated as though they have a high clinical suspicion of TB (i.e. treatment is initiated in all patients on the basis of clinical judgment).

These additional scenarios are considered to be extreme cases. NAAT is expected to be most cost-effective in the TB low scenario, as it is associated with more benefits in those considered to have a low clinical suspicion of TB. In contrast, NAAT is also expected to be least cost-effective in the scenarios in which all patients with TB, and with or without TB, respectively, are managed as though they have a high clinical suspicion of TB. In these scenarios treatment initiation decisions are based on clinical judgement, with the benefit of NAAT restricted to identifying drug resistance to initiate an appropriate treatment earlier. In the perfect clinical judgement scenario all true TB-negative patients are treated as though they have a low clinical suspicion of TB, and so treatment decisions are based on the results of AFB ± NAAT and only false-positive patients will receive treatment (determined by the specificity of testing). The relative cost-effectiveness of NAAT between these extreme high and low scenarios is likely to be determined by the relative specificity of NAAT compared with AFB.

The cost-effectiveness of the TB mixed scenario, which is thought best to reflect current practice, is likely to lie between the extreme additional scenarios.

Model assumptions

- When AFB and NAAT are discordant, the treatment decision is based on NAAT (consistent with PASC protocol)

- C&S testing (the reference standard) is assumed to be 100% sensitive and specific, as all patients have C&S testing and at the end of 2 months all will have correct diagnosis (i.e. MDR-TB, TB or no TB)
- To simplify the model structure, rifampicin resistance is used as a surrogate marker of MDR-TB (Lumb 2000), as the majority (37/40) of Australian bacteriologically confirmed cases in 2010 with rifampicin resistance were also MDR (Lumb et al. 2013)
- Once the decision to initiate or delay treatment has been made, the model assumes there will be no change in treatment until the results of C&S are available; this assumption may favour NAAT, as the earlier initiation of resistant drugs in the comparator arm would reduce the benefit of introducing NAAT
- Cost and utility penalties associated with the secondary transmission of TB are applied for each index case in the model, but the consequences (cost or health outcome) of further ongoing transmissions (e.g. tertiary transmissions and beyond) are not included in the base-case.

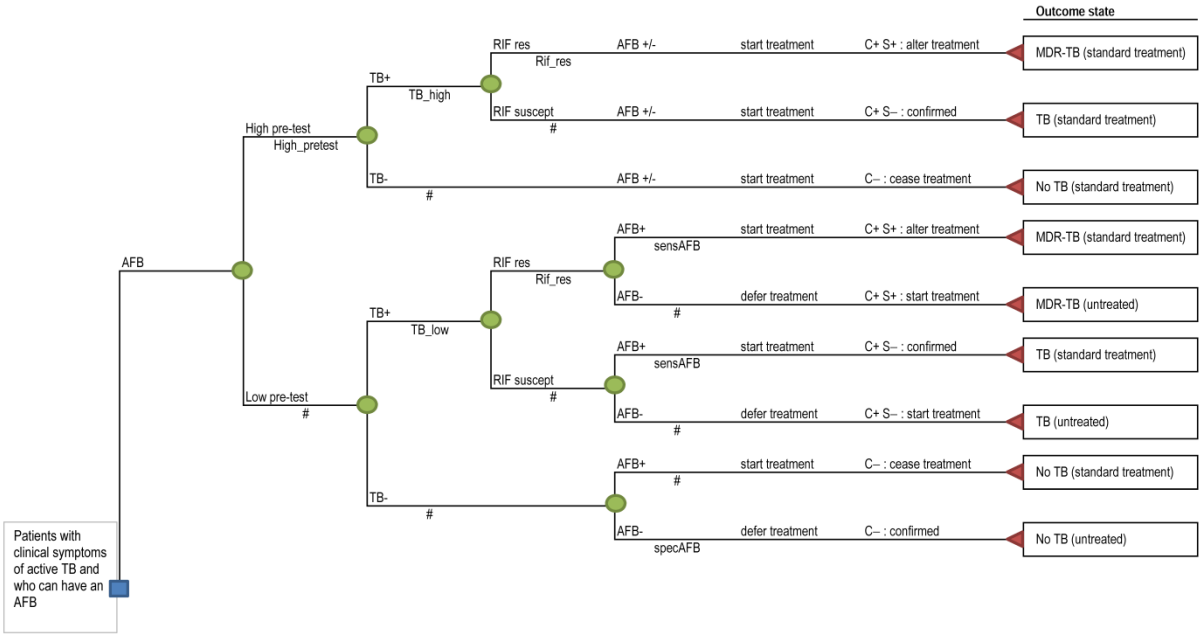

Figure 33 Decision analytic structure of the economic evaluation, comparator (AFB) model arm

AFB = acid-fast bacilli test; C = culture; High_pretest = proportion of patients considered to have high clinical suspicion of TB; MDR = multidrug-resistant; RIF res = rifampicin resistant; RIF suscept = rifampicin susceptible; Rif_res = prevalence of rifampicin resistance in TB; sensAFB = sensitivity of AFB for TB; specAFB = specificity of AFB for TB; S = susceptibility; TB = tuberculosis; TB_high = prevalence of TB in high clinical suspicion population; TB_low = prevalence of TB in low clinical suspicion population

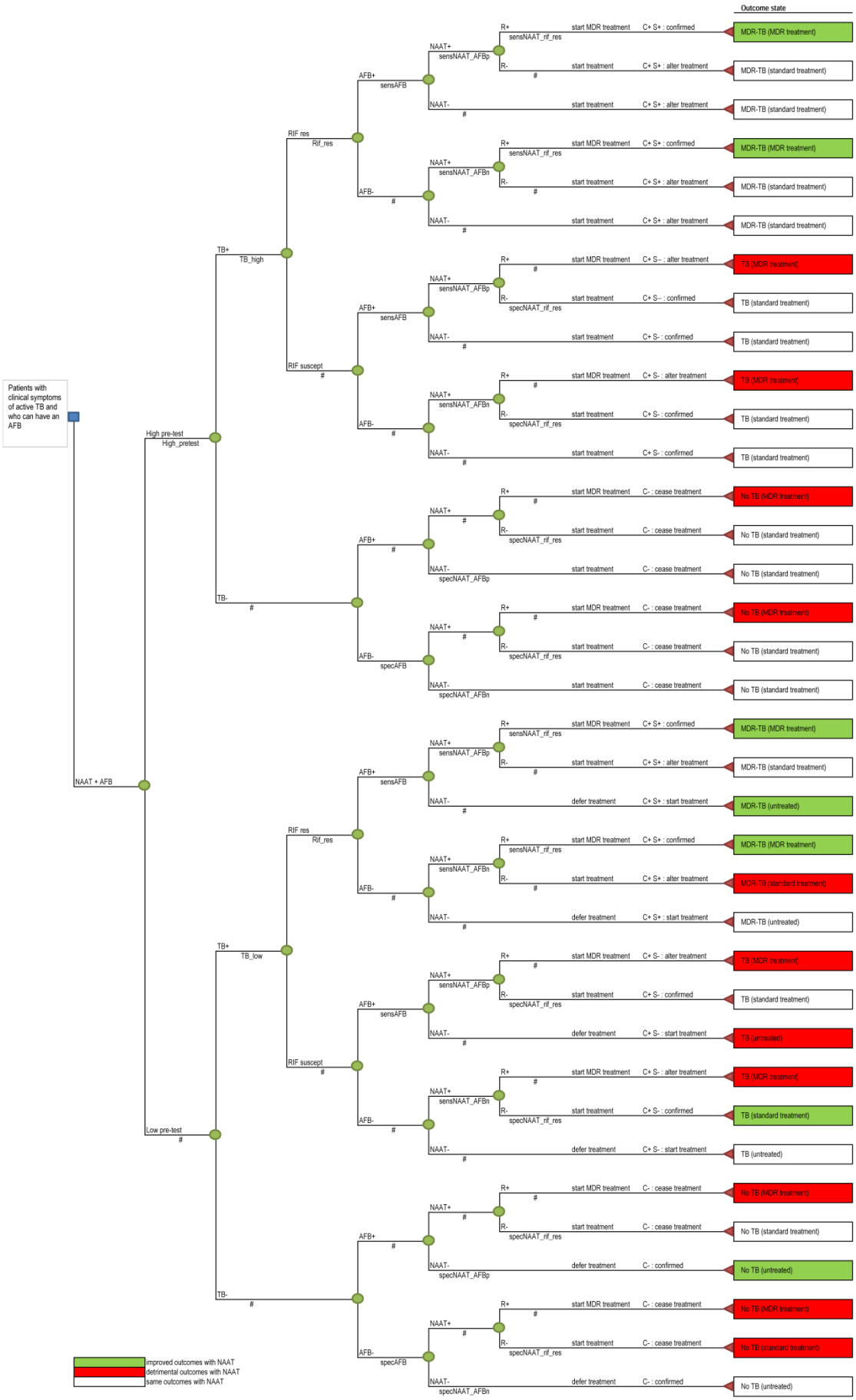

Figure 34 Decision analytic structure of the economic evaluation, intervention (AFB plus NAAT) model arm

AFB = acid-fast bacilli test; C = culture; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; R = resistance; RIF res = rifampicin resistant; RIF suscept = rifampicin susceptible; Rif_res = prevalence of rifampicin resistance in TB; sensAFB = sensitivity of AFB for TB; sensNAAT_AFBn = sensitivity of NAAT for TB in AFB-negative; sensNAAT_AFBp = sensitivity of NAAT for TB in AFB-positive; sensNAAT_rif_res = sensitivity of NAAT for rifampicin resistance; specAFB = specificity of AFB for TB; specNAAT_AFBn = specificity of NAAT for TB in AFB-negative; specNAAT_AFBp = specificity of NAAT for TB in AFB-positive; specNAAT_rif_res = specificity of NAAT for rifampicin resistance; S = susceptibility; TB = tuberculosis; TB_high = prevalence of TB in high clinical suspicion population; TB_low = prevalence of TB in low clinical suspicion population

Implications for false-positive and false-negative results

The decision trees presented in Figure 33 and Figure 34 culminate in nine different categories according to whether a true or false result is initially concluded (referred to as 'outcome states'). These are summarised in Table 44.

Table 44 Summary of decision tree outcome states in the economic evaluation

True status	Treated status	Implication
No TB	Untreated (TBTN)	Correct no treatment
	Standard treatment (TBFP, TRN)	Standard treatment initiated, stop treatment on C&S results
	MDR treatment (TBFP, FRP)	MDR treatment initiated, stop treatment on C&S results
TB	Untreated (TBFN)	No treatment initiated, begin standard treatment on C&S results
	Standard treatment (TBTP, TRN)	Correct standard treatment
	MDR treatment (TBTP, FRP)	MDR treatment initiated, switch to standard treatment on C&S results
MDR-TB	Untreated (TBFN)	No initial treatment initiated, begin MDR treatment on C&S results
	Standard treatment (TBTP, FRN)	Standard treatment initiated, switch to MDR treatment on C&S results
	MDR treatment (TBTP, TRP)	Correct MDR treatment

C&S = culture and sensitivity; FRN = false resistance negative; FRP = false resistance positive; MDR = multidrug-resistant; TB = tuberculosis; TBFN = tuberculosis false negative; TBFP = tuberculosis false positive; TBTN = tuberculosis true negative; TBTP = tuberculosis true positive; TRN = true resistance negative; TRP = true resistance positive

False-negative results (i.e. initially untreated TB (± MDR) or initial standard treatment in MDR-TB)

As there was no indication from the clinical assessment that a treatment delay of up to 2 months leads to an increase in disease severity (van der Oest, Kelly & Hood 2004), the economic modelling will assume treatment duration and QoL (from the time of correct diagnosis) as for those correctly treated. However, there is some indication that a delay in treatment leads to an increased risk of TB transmission (Ponticiello et al. 2001). A cost and utility penalty are applied to account for the treatment costs and utility decrement associated with secondary infections; see 'TB transmissions' and Utility penalty for active TB transmissions' for further details.

Treatment outcomes in MDR-TB patients treated initially with the standard regimen are assumed to be poorer than for those initially untreated, as treatment is ineffective and

associated with AEs (i.e. outcomes equal to those untreated who then have a disutility associated with treatment applied).

False-positive results (i.e. initial TB (± MDR) treatment in true-negative patients or MDR-TB treatment in susceptible TB)

Patients that are truly negative for TB who undergo initial TB (± MDR) treatment are assumed to have the cost and disutility of 2 months of the applicable treatment applied. As these patients may have a range of alternative diagnoses that present with similar symptoms (associated with differing costs and outcomes), the delay to treatment for the alternative diagnosis is not considered in the assessment.

Patients with true susceptible TB that are treated initially with MDR regimen are assumed to be effectively treated but have poorer overall health outcomes than those treated with the standard regimen because of the increased AEs associated with MDR treatment.

Inputs to the economic evaluation

Prevalence

Prevalence of TB

The prevalence of TB, defined as culture-positive, in studies included for the diagnostic accuracy of NAAT conducted in a low TB incidence country (k=11) was 24% (range 6–60%) (Table 93, Appendix D).

A reliable estimate for the prevalence of TB in the population with clinical signs and symptoms of active TB in Australia was not identified during the assessment, nor were estimates of the respective prevalences where that patient group is divided into those considered to have either a high or low clinical suspicion of TB. This introduces considerable uncertainty in the economic modelling, as the cost-effectiveness is likely to be sensitive to these variables.

Given the uncertainties in prevalence estimates identified, further information was provided by the applicant (an Australian pathology provider)[20]. It was estimated that 10–20% of patients would be considered to have a high clinical suspicion of TB, of which 50–70% would have TB. In those considered to have a low clinical suspicion of TB (the remaining 80–90% of patients) the prevalence is estimated to be in the range 5–10%. Using the upper limits of

[20] Personal communication from applicant via Department of Health, received 25 September 2014

these estimates provides an overall prevalence estimate of 22% (Table 45). This value is reasonably similar to the prevalence of TB reported in the diagnostic accuracy studies conducted in low incidence countries (24%, Table 93, Appendix D), and so appears to have face validity.

It would be expected that the higher the proportion of patients with true TB that are treated based on clinical judgement, the less cost-effective NAAT will be, as there are fewer benefits of NAAT for patients managed this way; therefore, using the upper limit of these estimates is the conservative choice and will be used in the base-case analysis of the economic evaluation. However, it should be noted that if the overall prevalence of TB is an overestimate, the cost-effectiveness of NAAT may too be overestimated. Given that these are best-guess estimates, sensitivity analyses around these estimates will be presented.

Additional scenarios are presented to examine the extent to which treatment initiation decisions based on clinical suspicion affect the ICER. The base-case prevalence of 22% is maintained in these scenarios; however, all are managed as though they have either low or high clinical suspicion of TB, depending on the scenarios (Table 45).

Table 45 Prevalence estimates used in previously published economic evaluations of NAAT

Scenario	Proportion high clinical suspicion [A]	Prevalence (high clinical suspicion)[B]	TB high clinical suspicion [C] (A × B)	Prevalence (low clinical suspicion) [D]	TB low clinical suspicion [E] ((1 – A) × D)	Total (C + E)
TB mixed	20%	70%	14%	10%	8%	22%
TB low suspicion	0%	0%	0%	22%	22%	22%
Perfect clinical judgment	22%	100%	22%	0%	0%	22%
TB high suspicion	100%	22%	22%	0%	0%	22%

NAAT = nucleic acid amplification testing; TB = tuberculosis

Prevalence of MDR-TB

The Australian Mycobacterium Reference Laboratory Network reported 1,051 bacteriologically confirmed cases of TB in 2010 (Lumb et al. 2013). Results of susceptibility testing to first-line treatments were available for 1,050 cases (99.9%) and multidrug resistance was reported in 37 cases (3.5%). However, 16 patients with MDR-TB were Papua New Guinea nationals who accessed health services in the Torres Strait Protection Zone. The remaining 21 MDR-TB patients were people who lived in Australia. This represents 2.0% of the bacteriologically confirmed cases of TB. This estimate will be used as the base-case estimate for the prevalence of MDR-TB in TB cases in the economic analysis. An upper limit of 3.5% will be tested in sensitivity analyses, to reflect the proportion of MDR-TB in all bacteriologically confirmed cases of TB in 2010; a lower limit of 0.5% will be used, which

reflects the lowest proportion observed by the Australian Mycobacterium Reference Laboratory Network since 1995 (Figure 35). This is consistent with proportions observed in Victoria during 2002–07 (0.6%–2.2%) (Lavender, Brown & Johnson 2009).

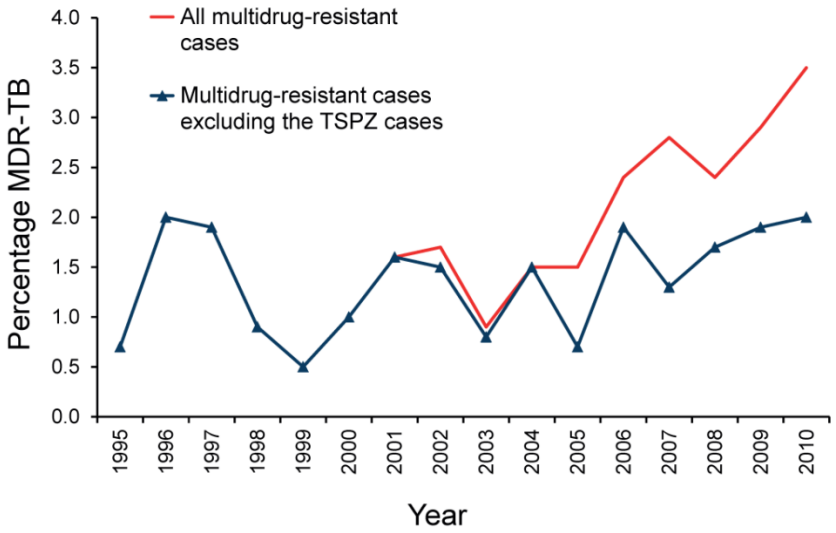

Figure 35 Percentage of TB cases that exhibited multidrug resistance in Australia, 1995–2010
MDR-TB = multidrug-resistant tuberculosis; TSPZ = Torres Strait Protection Zone
Source: Figure 2, Lumb et al. (2013)

Test parameters

As NAAT is an add-on test, and as accuracy of NAAT differs by AFB result, accuracy estimates of NAAT used in the model are separated by AFB status.

Accuracy estimates used in the economic evaluation are based on the results of the meta-analyses of all patients, in all tissue types, as presented in the clinical assessment (Table 46). As accuracy estimates reported in studies that were conducted in low-incidence countries (Canada, France etc.) may be more applicable to the Australian context, these estimates will be used in the base-case analysis, with 95%CI tested in sensitivity analyses. Given that countries in a low-incidence setting form the minority of results for the accuracy of AFB (k=11) and NAAT by AFB status (k=4), sensitivity analyses will be presented using the results for these parameters from all studies included in the clinical assessment.

Table 46 Test parameters used in the economic evaluation

Test	k	Sensitivity [95%CI]	Specificity [95%CI]	Source
Base case (low-incidence countries)				
AFB for TB	11	56% [44, 68]	98% [94, 100]	Figure 51
NAAT in AFB+ for TB	4	98% [94, 100]	97% [1, 100]	Figure 51
NAAT in AFB– for TB	4	70% [51, 84]	99% [94, 100]	Figure 51
NAAT for rifampicin resistance	8	92% [81, 97]	99% [96, 100]	Figure 51

Test	k	Sensitivity [95%CI]	Specificity [95%CI]	Source
Sensitivity analyses (all countries)				
AFB for TB	68	62% [54, 69]	98% [97, 99]	Figure 51
NAAT in AFB+ for TB	25	99% [96, 100]	78% [53, 92]	Figure 51
NAAT in AFB– for TB	39	80% [69, 87]	94% [88, 97]	Figure 51
NAAT for rifampicin resistance	11	93% [85, 97]	91% [78, 96]	Figure 51

AFB = acid-fast bacilli test; NAAT = nucleic acid amplification test; TB = tuberculosis

Healthcare resources

Test costs

The PASC protocol does not provide a proposed item fee for NAAT but indicates that the New South Wales (NSW) Mycobacterium Reference Laboratory charges $200 per NAAT, while that in Victoria charges $88. Both these laboratories were contacted during the assessment to confirm these costs and seek further information that may explain the differences in cost (e.g. commercial versus in-house, or if separate tests are conducted for resistance mutation testing). The Victorian laboratory indicated that an in-house NAAT costs $82 and that using the commercial Xpert kit is $130, met primarily through the Victorian State Government—only private patients & non-Australian residents are billed for testing[21]. It is unclear if the in-house PCR cost includes that of *rpoB* sequencing. This laboratory also indicated that rifampicin resistance mutations identified using Xpert are confirmed by in-house *rpoB* sequencing before results are released. These costs are assumed to be additional to the $130 test cost. No further information was provided by the NSW laboratory.

A search of pathology providers across the country indicated that at least two NSW public (bulk-billing) services[22] bill 'TB-PCR' under MBS item 69494 ($28.65). It is unclear if this is indicative of the cost of NAAT for TB, or if it is used as a partial subsidy and the NSW State Government is responsible for the difference. A private pathology provider in Victoria charges $100.50 for '*Mycobacterium* TB-PCR' with no Medicare funding[23].

The Mycobacterium Reference Laboratories in the other states (South Australia (SA), Western Australia (WA) and Queensland) were also contacted during the assessment to

[21] Personal communication, received 1 October 2014

[22] Sydney South West Pathology Service (www.sswahs.nsw.gov.au/sswps) and Pathology North (http://www.palms.com.au/php/labinfo/info_index.php?tc=MYCPCR&site=RNSH&tn=Mycobacteria%20PCR&s=Induced%20Sputum&sid=59)

[23] Melbourne Pathology http://mpscsp1.sonichealthcare.com.au/pseudompp/tcm/csp/searchview.csp

gather information regarding current NAAT use and costs. The laboratory in SA indicated that they conduct NAAT using the commercial Xpert kit at a cost of $70, which is currently funded by the SA State Government[24]. In WA, NAAT is conducted using either the Xpert kit or in-house real-time PCR (the choice of which depends on microscopy result, specimen type and clinical history), with an approximate cost of $40 per test, met predominantly by the laboratory and/or public health authorities[25].

The applicant has indicated that they are charged approximately $100 by their state reference laboratory; however, the applicant assumes that this cost includes NAAT in addition to TB antigen and high-performance liquid chromatography testing, and so the approximate cost is not indicative of NAAT alone[26].

In the absence of further information regarding NAAT costs, the base-case analysis assumes a test cost of $130 (based on the Victorian reference lab Xpert cost, as per advice from the Department of Health Policy Area). ICERs using alternative item fees for NAAT are presented in Appendix J.

As diagnostic AFB and C&S testing applies to all patients in both model arms, costs associated with these tests will not be considered.

Treatment costs

Costs were sourced for medications used commonly to treat susceptible TB and MDR-TB (Street et al. 2012). Sources included the Pharmaceutical Benefits Schedule (PBS), where listed, and Chemist Warehouse, where not listed on the PBS. However, not all medications used in the treatment of TB are marketed for use in Australia, and so are only available through the Special Access Scheme. In these instances, and where costs could not be sourced alternatively, they were sourced from a public hospital pharmacy[27]. Some medications require co-administration with pyridoxine; these costs have been included in the analysis.

For the treatment of susceptible TB, the standard regimen consists of 2 months' treatment with isoniazid, rifampicin, pyrazinamide and ethambutol (intensive phase), followed by a

[24] Personal communication, received 1 September 2014

[25] Personal communication, received 4 November 2014

[26] Personal communication from applicant via Department of Health, received 18 August 2014

[27] The hospital pharmacy department requested that it not be identified for reasons of commercial confidence

further 4 months with isoniazid and rifampicin (continuation phase). Daily doses are assumed based on the maximum dose per day (Street et al. 2012).

For the treatment of MDR-TB it is assumed that the organism is resistant to isoniazid and rifampicin. The intensive phase of treatment consists of pyrazinamide, ethambutol, amikacin, moxifloxacin and prothionamide for 6 months, followed by 12 months of pyrazinamide, ethambutol, moxifloxacin and prothionamide (Street et al. 2012). Amikacin is initially given intravenously via a peripherally inserted central catheter 5 days per week for the first 3 months and 3 days per week for the following 3 months (Jenkins, Dedicoat & Cook 2013). A one-off catheterisation cost has been applied in the model to account for the insertion of the catheter (MBS item 13815, $85.25). After the initial hospitalisation period (see 'Hospitalisation'), administration is assumed to occur in the home (Jenkins, Dedicoat & Cook 2013) at a cost of $234 per administration (Victoria State Government Department of Health 2014) (see Table 102, Appendix I).

Costs per month have been calculated and are presented in Table 47. These costs are assumed to apply each month while on treatment.

Table 47 Resource items associated with treatment of TB used in the economic evaluation

Type of resource item	Natural unit of measurement	Unit cost	Source of unit cost	Cost per month
Medication (daily dose)				
Isoniazid (300mg)	100mg tablet, 100 pack	$21.83	PBS item 1554T	3 tablets/day = 0.91 packs/month: $19.93
Rifampicin (600mg)	300mg capsule, 100 pack	$147.98	PBS item 1983J	2 capsules/day = 0.61 packs/month: $90.08
Pyrazinamide (2000mg)	500mg tablet, 100 pack	$77.00	Public hospital pharmacy [a]	4 tablets/day = 1.22 packs/month: $93.75
Ethambutol (1200mg)	400mg tablet, 56 pack	$133.99	Chemist Warehouse [b]	3 tablets/day = 1.63 packs/month: $218.48
Amikacin (571mg)	500mg/2 mL vial, 5 vial pack	$470.24	Public hospital pharmacy [a]	1.14 vials/day = 6.95 packs/month: $3,269.07
Moxifloxacin (400mg)	400mg tablet, 5 pack	$72.99	Chemist Warehouse [d]	1 tablet/day = 6.09 packs/month: $444.33
Prothionamide (750mg)	250mg tablet, 100 pack	$304.86	Public hospital pharmacy [a]	3 tablets/day = 0.91 packs/month: $278.38
Associated costs				
Pyridoxine with isoniazid (25mg)	25mg tablet, 100 pack	$7.99	Chemist Warehouse [e]	1 tablet/day = 0.30 packs/month: $2.43
Pyridoxine with prothionamide (300mg)	100mg tablet, 50 pack	$11.02	Chemist Warehouse [f]	3 tablets/day = 1.83 packs/month: $20.13

Type of resource item	Natural unit of measurement	Unit cost	Source of unit cost	Cost per month
Peripherally inserted central catheter (amikacin administration)	Insertion	$85.25	MBS item 13815	One-off cost (first-month only)
Amikacin administration	Per infusion	$234.00	Victorian State Government (2014)	Total administration cost for false-positive MDR results: $8,002 Total administration cost for true MDR-TB: $20,943 (see Table 102, Appendix I)

[a] personal communication
[b] http://www.chemistwarehouse.com.au/product.asp?id=61386&pname=Myambutol+400mg+Tablets+56 (accessed 12 September 2014)
[c] Assuming 1000mg dose 5 days per week for 3 months, and 1000mg dose 3 times per week for 3 months (Jenkins, Dedicoat & Cook 2013)
[d] http://www.chemistwarehouse.com.au/product.asp?id=55677&pname=Avelox+400mg+Tablets+5 (accessed 12 September 2014)
[e] http://www.chemistwarehouse.com.au/product.asp?id=7339&pname=Pyroxin+Tablets+25mg+100 (accessed 12 September 2014)
[f] http://www.chemistwarehouse.com.au/product.asp?id=7340&pname=Pyroxin+Tablets+100mg+50 (accessed 12 September 2014)

The cost per month by treatment regimen (standard or MDR) and phase (intensive or continuing) is presented in Table 48.

Table 48 Cost per treatment regimen, per month

Treatment regimen	Consists of	Cost per month
Standard, IP	Isoniazid [a], rifampicin, ethambutol, pyrazinamide	$425
Standard, CP	Isoniazid [a], rifampicin	$112
MDR, IP	Ethambutol, pyrazinamide, amikacin, moxifloxacin, prothionamide [a]	$4,324
MDR, CP	Ethambutol, pyrazinamide, moxifloxacin, prothionamide [a]	$1,055

[a] Co-administered with pyridoxine
CP = continuation phase; IP = intensive phase; MDR = multidrug-resistant

The total treatment course cost by outcome state is presented in Table 49.

Table 49 Total months in treatment and regimen costs, by outcome state

True status	Treated status	No treatment	Standard (IP) (months)	Standard (CP) (months)	MDR (IP) (months)	MDR (CP) (months)	Treatment course cost a
No TB	Untreated	20	0	0	0	0	$0
	Standard treatment	18	2	0	0	0	$849
	MDR treatment	18	0	0	2	0	$16,735
TB	Untreated	14	2	4	0	0	$1,299
	Standard treatment	14	2	4	0	0	$1,299
	MDR treatment	12	2	4	2	0	$18,035
MDR-TB	Untreated	2	0	0	6	12	$59,232
	Standard treatment	0	2	0	6	12	$60,081
	MDR treatment	2	0	0	6	12	$59,332

[a] Calculated by multiplying the duration by the per-month treatment cost (including one-off cost for insertion of catheter for amikacin and administration costs). For example, the total treatment cost for untreated TB is equal to the sum of 6 months of no treatment (no cost), 2 months of standard intensive treatment (2 × $425) and 4 months of standard continuation treatment (4 × $112), which equals $1,299 (may not be exact due to rounding). Treatment costs are discounted at 5% per year when accrued beyond 1 year.
CP = continuation phase; IP = intensive phase; MDR = multidrug-resistant; TB tuberculosis

Costs of treating AEs associated with TB treatment.

The proportion of patients who experience an AE while on treatment is assumed to differ depending on the treatment regimen administered, as drugs commonly used in the treatment of MDR-TB are poorly tolerated (Street et al. 2012). Francis et al. (2014) conducted a retrospective case-control study of MDR-TB patients matched to susceptible TB patients for site of TB, HIV status, age and sex. AEs were reported for each group (Table 50); however, the severity and treatment of AEs were not reported.

The model assumes the same AE management for all patients who experience the same AE, with treatment decisions based on Victorian guidelines for the management of TB (Street et al. 2012). Further, it is also assumed that AEs would be experienced while in the intensive phase of treatment, and so the costs of treating AEs (as per Table 50) are applied accordingly. For example:

- A false MDR-TB-positive patient (i.e. false-positive results for TB and resistance) is assumed to experience AEs related to MDR treatment, and so will have the cost ($34.29) applied
- As an MDR-TB patient on standard treatment (i.e. true-positive TB result, false-negative result for resistance) has 2 months of intensive standard treatment followed by the appropriate MDR regime, these patients are assumed to experience AEs associated with both standard and MDR treatment.

AEs that are managed by either altering doses or stopping treatment (i.e. temporary or permanent) have not been costed. These include hearing impairment, tinnitus and visual disturbances.

Table 50 Cost of treating AEs, by treatment regimen

AE	Treatment	Proportion TB	Proportion MDR-TB	Treatment cost	Source	TB	MDR-TB
Arthralgia	Ibuprofen	1/48 (2%)	0/16 (0%)	$14.87	PBS 3192B	$0.31	$0.00
Hypothyroidism	Thyroxine	0/48 (0%)	1/16 (6%)	$29.66	PBS 2175L	$0.00	$1.85
Nausea/vomiting	Cimetidine	5/48 (10%)	11/16 (69%)	$22.45	PBS 1158Y	$2.34	$15.43
Psychiatric problems	Haloperidol	0/48 (0%)	7/16 (44%)	$16.24	PBS 2761H	$0.00	$7.11
Rash/itch	Loratidine	10/48 (21%)	2/16 (13%)	$46.26	PBS 4313B	$9.64	$5.78
Renal dysfunction	Replace electrolytes	0/48 (0%)	1/16 (6%)	$65.81	PBS 3117C, 1841X, 5146W	$0.00	$4.11

AE	Treatment	Proportion TB	Proportion MDR-TB	Treatment cost	Source	TB	MDR-TB
TOTAL						$12.29	$34.29

AE = adverse events; MDR = multidrug-resistant; TB = tuberculosis

TB management costs

Management of patients treated for TB is costed based on Victorian guidelines for the management of TB (Street et al. 2012). The type of health resource item, frequency of use, and overall use and costs by outcome state are presented in Table 51.

Hospitalisation

Hospital isolation after diagnosis of TB is important to contain the spread of the disease. The costs associated with hospital isolation used in the economic evaluation are presented in Table 52. Francis et al. (2014) report the proportion of Western Australian MDR-TB patients and susceptible TB controls (matched for site of TB, HIV status, age and sex) that were hospitalised during treatment and the mean total days in hospital. It was observed that significantly more patients with MDR-TB (100%) were hospitalised for an average of 26 days, compared with 35% of those with susceptible TB, who were hospitalised on average for 13 days ($p<0.001$). Sensitivity analyses will be conducted around these estimates.

To estimate the average cost of hospital isolation, National Hospital Costing Data have been used (Round 14, 2009–10) (Australian Government Department of Health 2012). The average total cost per Respiratory Tuberculosis DRG (E76Z) in a public hospital was $14,230, including $904 for pharmacy costs. The average length of stay was 14.6 days. Excluding pharmacy costs (as these are costed elsewhere), the average cost per hospitalised day is $914[28]. A standardised growth rate of 2.6% is applied to estimate the cost in 2014 dollars ($1,039) (Independent Hospital Pricing Authority 2014). These costs are applied to all patients with TB (± MDR) on diagnosis (immediate or delayed), as it is assumed that even if diagnosis is delayed, the same level of hospitalisation is applied for isolation and treatment once a contagion risk has been identified.

[28] ($14,230−$904)/14.58

Table 51 Resource use associated with the management of TB used in the economic evaluation, by outcome state, discounted (where appropriate)

Type of resource item	Frequency of use	Unit cost	Source of unit cost	No TB, unTx	No TB, Std	No TB, MDR	TB, unTx	TB, Std	TB, MDR	MDR, unTx	MDR, Std	MDR, MDR
Specialist attendance	At 2 weeks, then monthly for duration of treatment	$43.00	MBS 105	0	3	3	7	7	9	19	21	19
Visual acuity [a]	At baseline, and during specialist attendance, while on ethambutol	N/A	N/A	0	4	4	4	4	4	20	22	20
MC&S	At 2 weeks, then monthly for duration of treatment (MDR treatment: after 6 months, quarterly)	$43.00	MBS 69324	0	3	3	7	7	9	11	13	11
Chest X-ray	Quarterly	$47.15	MBS 58503	0	0	0	2	2	3	6	7	6
Full blood examination	Baseline	$16.95	MBS 65070	0	1	1	1	1	1	1	1	1
Erythrocyte sedimentation rate	Baseline	$7.85	MBS 65060	0	1	1	1	1	1	1	1	1
Liver function tests	Baseline (MDR: fortnightly for first month, monthly for duration)	$17.70	MBS 66512	0	1	4	1	1	4	20	20	20
Urea and electrolytes	Baseline (amikacin: at 2 weeks, then monthly for duration of treatment)	N/A [b]	N/A	0	1	4	1	1	4	8	8	8
Calcium and magnesium	Monthly while on amikacin	N/A [b]	N/A	0	0	2	0	0	2	6	6	6
Amikacin trough levels	At 2 weeks, then monthly for duration of amikacin treatment	$18.15	MBS 66800	0	0	3	0	0	3	7	7	7
Audiometry	Baseline and 2-monthly while on amikacin	$21.90	MBS 11306	0	0	2	0	0	2	4	4	4
Thyroid function tests	Quarterly while on prothionamide	$34.80	MBS 66719	0	0	1	0	0	1	6	6	6
TOTAL				$0	$301	$487	$739	$739	$1,144	$2,334	$2,553	$2,346

[a] Costed as part of specialist attendance
[b] Ordered at same time as liver function tests, no additional cost as tests also listed in MBS item 66500
MC&S = AFB microscopy, culture and sensitivity; MDR = multidrud-resistant; Std = standard treatment; TB = tuberculosis; unTx = untreated

Table 52 Total cost of hospital isolation

	Susceptible TB	MDR-TB
Proportion isolated	35%	100%
Days isolated (range)	13 (2–41)	26 (1–99)
Cost per day hospitalised	$1,039	$1,039
Total cost	$4,728	$27,018

MDR-TB =multidrug-resistant tuberculosis; TB = tuberculosis

In patients with TB hospitalisation, costs are assumed to apply by true status. As there was no indication from the clinical evidence that a delay in diagnosis of 2 months leads to inferior outcomes such as longer treatment duration or hospitalisation, true-positive and false-negative TB patients are assumed to have the same hospitalisation costs applied (despite the accrual of costs at differing times).

For patients with a false TB diagnosis, duration of hospitalisation is assumed as for susceptible TB (as AFB microscopy after two weeks will likely be negative).

TB transmissions

The costs associated with TB transmission can be separated into those associated with (i) screening contacts and (ii) treatment of contacts identified with either latent or active TB. Consistent with evidence identified in the clinical assessment, patients in whom treatment is delayed are assumed to infect more contacts than those treated earlier (Ponticiello et al. 2001).

Ponticiello et al. (2001) report that the 90 TB patients enrolled in their study had 346 contacts screened (average 3.84 per patient). However, the study did not report the drug-resistance status of patients. It is unclear whether the number of contacts screened would be similar between patients with susceptible TB and MDR-TB. A retrospective analysis conducted in Canada (Johnston et al. 2012) observed no significant difference in the median number of contacts screened per case of susceptible TB (cases: n=2,895; contacts: n=7,309) or MDR-TB (cases: n=28; contacts: n=89), with a median of 3 contacts per case reported (p=0.839). This is in contrast to a median of 6 contacts per case of MDR-TB (cases: n=16; contacts: n=727) and 3 per case of susceptible TB (cases: n=48; contacts: n=371) reported in the retrospective case-control study of Western Australian patients conducted by Francis et al. (2014). The Australian data will be used in the base-case analysis of the economic evaluation.

Ponticiello et al. (2001) observed that 6/43 (14%) contacts of cases with a delay to treatment of less than 1 month had a latent TB infection, and 24/56 (43%) contacts of cases

with a delay of treatment of 2 months had latent TB. As this study did not report the drug-resistance status of patients, and as no evidence was identified in the clinical assessment for the effect of delayed treatment in MDR-TB, assumptions regarding latent MDR-TB transmission have been made in the modelling. The transmissibility of MDR-TB relative to susceptible TB has been reported to vary substantially—more infectious in some studies and less infectious in others (Borrell & Gagneux 2009). A conservative approach is taken in the base-case analysis of the economic evaluation, which assumes a poor relative infectivity of MDR-TB (30%) (Cohen & Murray 2004), as any overestimation of the transmissibility of MDR-TB will overestimate the costs of MDR-TB transmissions, disproportionately affecting the comparator. This is due to all patients with MDR-TB receiving ineffective treatment under current testing, and so remaining infectious, until the C&S results. This will be tested in the sensitivity analyses.

Ponticiello et al. (2001) report that 18/125 (14%) contacts with a latent TB infection developed active TB during follow-up. This was not reported by the delay to treatment in the index case, but has been estimated. The delay in treatment of 2 months compared with less than 1 month resulted in approximately three (43% vs 14%) times more latent TB infections, and this has been used to estimate the relative proportion of active infections in those with a delay in treatment (Table 53). The relative infectivity coefficient assumed for latent TB transmissions with MDR is also assumed to apply to the transmission of active MDR infections.

Contacts of index patients are screened using the tuberculin skin test (Mantoux test), which is listed on the MBS under item 73811 ($11.20); this test is performed at time of exposure and repeated 2–3 months later. Treatment of latent susceptible TB is according to Victorian guidelines for the management of TB (Street et al. 2012), and consists of 6 months' isoniazid treatment (Table 47). Treatment guidelines for latent MDR-TB were not identified, so treatment is assumed to consist of 6 months' moxifloxacin treatment, as per the most common treatment regimen reported of latent MDR-TB in a Victorian study conducted by Denholm et al. (2012) (6 months' fluoroquinolone) (Table 47). The cost of treating active infections includes treatment (and treatment of AEs), management and hospitalisations costs (Table 53).

The costs of baseline contact tracing only are assumed in contacts of false-positive TB patients.

Table 53 Total cost of identification and treatment of TB transmissions

		TB	MDR-TB	No TB	Delayed TB	Delayed MDR-TB
A	Contacts (Francis et al. 2014)	3	6	3	3	6
B	Tests per contact	2	2	1	2	2
C	Cost per TST (MBS item 73811)	$11.20	$11.20	$11.20	$11.20	$11.20
D	Fitness (relative to DS-TB)	1	0.30 [a]	0	1	0.30 [a]
E	Proportion with latent infection (Ponticiello et al. 2001)	(6/43) × D = 14%	(6/43) × D = 4%	0	(24/56) × D = 43%	(24/56) × D = 13%
F	No. of latent transmissions (A × E)	0.42	0.25	0	1.29	0.77
G	Latent infection regimen	Isoniazid	Moxifloxacin	N/A	Isoniazid	Moxifloxacin
H	Months of treatment	6	6	0	6	6
I	Cost per month of treatment (Table 47)	$22.37 [b]	$444.33	0	$22.37 [b]	$444.33
J	Treatment cost (G × H)	$134	$2,666	$0	$134	$2,666
K	Proportion of latent TB patients who develop active TB (Ponticiello et al. 2001)	18/125 (14%)	18/125 (14%)	0	18/125 (14%)	18/125 (14%)
L	By treatment delay	25% [c]	25% [c]	0	75% [d]	75% [d]
M	Proportion with active infection (D × K × L)	4%	1%	0%	11%	3%
N	No. of active transmissions (F × M)	0.015	0.003	0.000	0.140	0.025
O	Treatment cost (Table 49)	$6,778	$88,730	$0	$6,778	$88,730
	Cost penalty applied [e]	$224	$1,040	$34	$1,186	$4,422

[a] Cohen & Murray (2004)
[b] includes co-administration of pyridoxine
[c] 14%/(14% + 43%)
[d] 43%/(14% + 43%)
[e] (A × B × C) + (F × J) + (N × O)
DS-TB = drug-susceptible tuberculosis; MDR-TB = multidrug-resistant tuberculosis; TB = tuberculosis; TST = tuberculin skin test

Overall cost per outcome state

Total costs accrued over the 20-month time horizon, accounting for treatment, management, hospitalisation, transmissions and treatment of AEs, by outcome state, is presented in Table 54. These costs will be incorporated into the model in a stepped manner to view the effect of each on the resulting ICER.

It should be noted that these costs do not include the cost of NAAT, which applies to the intervention arm of the model only.

Table 54 Total costs, by outcome state, discounted (where appropriate)

True status	Treated status	Treatment	AEs	Management	Hospitalisation	Transmissions	TOTAL
No TB	Untreated	$0	$0	$0	$0	$0	$0
	Std treatment	$849	$12	$301	$4,728	$34	$5,924
	MDR treatment	$16,735	$34	$487	$4,728	$34	$22,018
TB	Untreated	$1,299	$12	$739	$4,728	$1,186	$7,965
	Std treatment	$1,299	$12	$739	$4,728	$224	$7,002
	MDR treatment	$18,035	$47	$1,144	$4,728	$224	$24,177
MDR-TB	Untreated	$59,232	$34	$2,334	$27,018	$4,422	$93,040
	Std treatment	$60,081	$47	$2,553	$27,018	$4,422	$94,121
	MDR treatment	$59,332	$34	$2,346	$27,018	$1,040	$89,771

Note: Costs associated with the correct treatment are highlighted.
AEs = adverse events; MDR = multidrug-resistant; Std = standard; TB = tuberculosis

Utility values

Utility values used in the previously published economic evaluations of NAAT are presented in Table 55.

The utility weights used in previously published cost–utility analyses of NAAT may be inappropriate to use in the current assessment, as a number of weights were found to be based on clinical opinion or assumptions, or could not be verified from the cited sources. To supplement these utility values, a search was conducted to identify studies that measure utility estimates in a TB population (see Appendix H). Six studies were identified that reported eliciting utility weights relevant to TB (Table 103, Appendix I).

Table 55 Utility values used in previously published economic evaluations of NAAT

Study	Utility weight		Comment
Choi et al. (2013)	Complete health First-line treatment (without TB) MDR-TB treatment (without TB) Treated active TB Untreated active TB Drug-related hepatotoxicity Death	1.0 0.9 0.7 0.85 0.7 0.8 0	Study refers to de Perio et al. (2009), which refers to Tsevat et al. (1988) for all utility weights Tsevat et al. (1988) states that values were assigned based on a consensus of internists and were assumed to be applicable to a US population
Hughes et al. (2012)	General population	0.86	EQ-5D weight elicited in general UK population (Kind, Hardman & Macran 1999)
	Decrement for active TB	0.39	Study refers to Tan et al. (2008), which cites Guo et al. (2008) (Table 103, Appendix I), but utility weight cannot be verified from source
	Decrement for treated active TB	0.1	Study cites Khan et al. (2002). Values were obtained from a panel of infectious-disease specialists with expertise in tuberculosis; utility weight cannot be verified from source

Study	Utility weight		Comment
	Decrement for toxicity with TB Decrement for toxicity without TB	0.25 0.16	Cites Holland et al. (2009) in which the utility of treatment-limiting toxicity in TB is based on an assumption

MDR-TB = multidrug-resistant tuberculosis; TB = tuberculosis

In the economic model, cases without TB are assumed to have a utility weight consistent with that of the general UK population (0.86), measured using the EQ-5D (Kind, Hardman & Macran 1999). The utility weights reported in Jit et al. (2011) (Table 103, Appendix I) are the most applicable to patients who have TB, as the study was conducted in the UK setting using the EQ-5D at diagnosis of TB (0.68) and after 2 months of treatment (0.81). These utilities are assumed in the model to apply to untreated and treated TB (± MDR). However, as the utilities were elicited after 2 months of standard treatment, this is assumed to apply to the continuation phase of treatment, and this estimate is assumed to not take into account disutility associated with treatment, including effects of AEs during the intensive phase.

To account for AEs associated with the intensive phase of treatment, in those with and without TB (i.e. false-positive patients), the utility weights for true-positive and false-positive treatment have a utility decrement applied. This decrement is estimated based on the utility decrement of toxicity with (0.22) or without (0.14) TB, adjusted[29] from those used in Hughes et al. (2012), and multiplied by the proportion of patients who experience AEs by MDR (81%) or standard (33%) treatment, as reported by Francis et al. (2014). These utilities are assumed to apply for each month while in the intensive phase of treatment (duration of 2 months in standard treatment and 6 months in MDR treatment).

Utility values used in the economic evaluation are presented in Table 56.

Table 56 Utility values used in the economic evaluation

Health state	Utility weight	QALYs accrued per month (utility weight/12)	Utility weight source/calculation
No TB or cured	0.86	0.072	Kind et al. (1999)
No TB, standard treatment	0.81	0.068	0.86 – (0.33 [a] × 0.14 [b])
No TB, MDR treatment	0.75	0.062	0.86 – (0.81 [c] × 0.14 [b])
TB, untreated	0.68	0.057	Jit et al. (2011)
TB, standard treatment (intensive phase)	0.74	0.062	0.81 – (0.33 [a] × 0.22 [d])

[29] Hughes et al. (2012) cites Holland et al. (2009), in which utility of no TB health state = 1 and a utility decrement of 0.25 is assumed for 'treatment-limiting toxicity'. As a utility of 0.86 is assumed in the model, a 25% reduction of 0.86 (0.22) is applied for toxicity associated with TB and a 16% reduction of 0.86 (0.14) for toxicity associated with no TB

Health state	Utility weight	QALYs accrued per month (utility weight/12)	Utility weight source/calculation
TB, MDR treatment (intensive phase)	0.64	0.053	0.81 – (0.81 [c] × 0.22 [d])
TB, treated (continuation phase)	0.81	0.068	Jit et al. (2011)
MDR-TB, standard treatment (intensive phase)	0.61	0.051	0.68 – (0.33 [a] × 0.22 [d])

[a] Proportion of patients who experience AEs with standard treatment, reported by Francis et al. (2014)
[b] Utility decrement of AEs related to TB treatment in patients without TB, as assumed in Hughes et al. (2012)
[c] Proportion of patients who experience AEs with MDR treatment, reported by Francis et al. (2014)
[d] Utility decrement of AEs related to TB treatment in true-positive patients, as assumed in Hughes et al. (2012)
AEs = adverse events; MDR = multidrug-resistant; TB = tuberculosis

Overall utility per outcome state

The overall utility accrued over the 20-month time horizon, accounting for time undiagnosed, time in each phase of treatment and time cured, by the decision tree outcome states is presented in Table 57.

Table 57 Overall utility, by outcome state, discounted (where appropriate)

True status	Treated status	Untreated TB (months)	Standard (IP) (months)	MDR (IP) (months)	Treated TB (CP) (months)	No TB or cured (months)	Total QALYs [a]
No TB	Untreated	0	0	0	0	20	1.406
	Standard treatment	0	2	0	0	18	1.398
	MDR treatment	0	0	2	0	18	1.387
TB	Untreated	2	2	0	4	12	1.339
	Standard treatment	0	2	0	4	14	1.369
	MDR treatment	0	2	2	4	12	1.332
MDR-TB	Untreated	2	0	6	12	0	1.216
	Standard treatment	0	2	6	12	0	1.204
	MDR treatment	0	0	6	12	2	1.245

Note: The outcomes associated with the correct treatment are highlighted.
[a] Calculated by multiplying the duration by the per-month utility weight. For example, the utility for untreated TB is equal to the sum of 2 months' untreated TB (2 × 0.057), 2 months' TB standard intensive phase (2 × 0.062), 4 months' TB continuation phase (4 × 0.068) and 12 months' cured (12 × 0.072), which equals 1.339 (figures not exact due to discounting of utilities accrued after 1 year and rounding)
CP = continuation phase; IP = intensive phase; MDR = multidrug-resistant; TB = tuberculosis

Utility penalty for active TB transmissions

To estimate the utility penalty for transmissions of active TB, it is assumed that these secondary patients receive the correct treatment according to the TB status of the index patient. For example, if an index patient had untreated MDR-TB, the secondary patient is assumed to have the 20-month utility of correctly treated MDR-TB. The utility difference between 20 months of no TB (1.406) and of correctly treated TB (± MDR) (1.369 or 1.245, respectively) is the penalty applied. The outcome-state utilities adjusted for TB

transmissions are presented in Table 58. Results will be presented both with and without the inclusion of these utility penalties.

No utility penalty is applied for the transmission of latent TB.

Table 58 Outcome state utilities, adjusted for TB transmissions

True status	Treated status	Index utility (Table 57)	Transmissions with active infection [a]	Utility penalty per transmission [b]	Weighted utility penalty [c]	Overall utility, adjusted for transmissions [d]
No TB	Untreated	1.406	0	0	0	1.406
	Standard treatment	1.398	0	0	0	1.398
	MDR treatment	1.387	0	0	0	1.387
TB	Untreated	1.339	0.33	0.037	0.012	1.327
	Standard treatment	1.369	0.11	0.037	0.004	1.365
	MDR treatment	1.332	0.11	0.037	0.004	1.328
MDR-TB	Untreated	1.216	0.20	0.161	0.031	1.184
	Standard treatment	1.204	0.20	0.161	0.031	1.172
	MDR treatment	1.245	0.06	0.161	0.010	1.235

Note: The outcomes associated with the correct treatment are highlighted.
[a] Row N, Table 53
[b] 20-month utility of no TB (untreated) – 20-month utility of correct TB (± MDR) treatment (Table 57)
[c] Transmissions with active infection × utility penalty
[d] Index utility – weighted utility penalty

Outputs from the economic evaluation

The results of the economic evaluation are presented for four scenarios:

- TB mixed scenario: patients with true TB are spread across treatment populations based on high or low clinical suspicion of TB (best reflective of current practice)
- TB low-suspicion scenario: all patients (including all with true TB) are treated as though they have a low clinical suspicion of TB—that is, clinical judgment is not used as a basis to initiate treatment but, rather, treatment decisions are based on AFB ± NAAT
- Perfect clinical judgment scenario: all patients with true TB are treated as per high clinical suspicion—that is, clinical judgement has 100% sensitivity and specificity in identifying TB; and all patients without TB are treated as per low clinical suspicion—that is, treatment decisions are based on AFB ± NAAT
- TB high-suspicion scenario: all patients are treated as though they have a high clinical suspicion of TB—that is, treatment is initiated in all patients on the basis of clinical judgment.

The following summarises the results of the economic evaluation that will be presented:

- disaggregated by decision tree probabilities, incremental costs and incremental outcomes (TB mixed scenario only); the disaggregated results for additional scenarios are presented in Appendix I
- for the TB mixed scenario, the incremental cost-effectiveness is presented incorporating costs in a stepped manner (additional scenarios in Appendix I)
- incremental cost-effectiveness of each of the three additional scenarios
- sensitivity analyses.

Outcome-state probabilities

The results of the decision tree analysis are presented in Figure 55 (AFB model arm) and Figure 56 (AFB plus NAAT model arm), Appendix I. The probability at each decision tree terminal is derived from a composite of the prevalence of TB in the tested population, clinical suspicion and test accuracy parameters. The difference between the model arms is the incorporation of NAAT accuracy parameters into the intervention arm.

The proportion of patients managed per each outcome state, as derived from the decision tree analysis, is presented in Table 59.

Table 59 Outcome state probabilities, TB mixed scenario

True status	Treated status	AFB	AFB + NAAT	Difference
No TB	Untreated	70.6%	71.3%	0.69%
	Standard treatment	7.4%	6.7%	–0.70%
	MDR treatment	0.0%	0.0%	0.01%
TB	Untreated	3.4%	1.1%	–2.33%
	Standard treatment	18.1%	20.3%	2.14%
	MDR treatment	0.0%	0.2%	0.18%
MDR-TB	Untreated	0.1%	0.0%	–0.05%
	Standard treatment	0.4%	0.1%	–0.30%
	MDR treatment	0.0%	0.3%	0.35%
TOTAL		100%	100%	0%

Note: The probabilities associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

The highlighted rows in Table 59 reflect the relevant optimal treatment strategy. NAAT is associated with improvements in each of these strategies, due to improved sensitivity of NAAT in conjunction with AFB (fewer false-negative results) and the ability of NAAT to identify and treat MDR-TB earlier. A net reduction in false-positive results is also observed, due to false-positive results associated with NAAT in AFB true-negative patients being outweighed by AFB false-positive patients correctly identified using NAAT.

Incremental costs

The incremental cost of NAAT, broken down by the source of the cost, is presented in Table 60. NAAT is associated with an incremental cost ($85.11) that is largely driven by the cost of NAAT, offset by reduced costs associated with TB transmissions (due to the identification of MDR-TB and fewer false-negative results) and reduced hospitalisations (due to fewer patients with false-positive results who require hospital isolation).

Table 60 Breakdown of incremental costs, TB mixed scenario

Cost	AFB	AFB + NAAT	Increment
Treatment	$607.05	$631.19	$24.14
Treatment of AEs	$3.76	$3.70	–$0.06
Management	$192.72	$190.79	–$1.93
Hospitalisation	$1,490.04	$1,457.36	–$32.68
TB transmissions	$103.40	$69.04	–$34.36
NAAT cost	$0.00	$130.00	$130.00
TOTAL	$2,396.97	$2,482.08	$85.11

AEs = adverse events; AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

Incremental QALYs

The incremental costs by outcome health state are derived from the product of the outcome-state probabilities (Table 59) and the overall utility, by outcome state as presented in Table 58. These are presented in Table 61. Overall NAAT is associated with a small incremental QALY gain (0.001), driven by a shift from initially untreated TB (or standard treatment in the case of MDR-TB) to correct treatment.

In part the small difference results from the assumption that the correct diagnosis is achieved in all patients after 2 months, and that this 2-month delay in treatment is not assumed to affect patient mortality rates or the severity of disease. Whereas a utility penalty has been applied for the transmission of active TB, none has been applied for the transmission of latent TB.

Table 61 Weighted utility by outcome state, TB mixed scenario

True status	Treated status	AFB	AFB + NAAT	Increment
No TB	Untreated	0.992	1.002	0.010
	Standard treatment	0.104	0.094	–0.010
	MDR treatment	0.000	0.000	0.000
TB	Untreated	0.046	0.015	–0.031
	Standard treatment	0.248	0.277	0.029
	MDR treatment	0.000	0.002	0.002

True status	Treated status	AFB	AFB + NAAT	Increment
MDR-TB	Untreated	0.001	0.000	–0.001
	Standard treatment	0.004	0.001	–0.004
	MDR treatment	0.000	0.004	0.004
TOTAL		1.395	1.396	0.001

Note: The outcomes associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Incremental cost-effectiveness

The effect on the ICER of adding each additional group of costs is presented in Table 62 for the TB mixed scenario. Consistent with Table 60, the addition of transmissions and hospitalisation costs contribute most to the final ICER. The addition of the utility penalty for transmissions decreases the ICER further. This effect is consistently observed in the additional scenarios (Table 113, Appendix I).

Table 62 Stepped economic evaluation, TB mixed scenario

Utilities considered	Costs included (NAAT cost applied in AFB + NAAT arm)	ICER
Index patient utility	Treatment only	$188,307
	Treatment and AEs	$188,238
	Treatment, AEs and management	$185,882
	Treatment, AEs, management and hospitalisation	$145,956
	Treatment, AEs, management, hospitalisation and transmission	$103,978
Index and secondary case utility	Treatment, AEs, management, hospitalisation and transmission	$90,728

AEs = adverse events; ICER = incremental cost-effectiveness ratio

The incremental cost-effectiveness of NAAT in each of the additional scenarios is presented in Table 63.

Table 63 Incremental cost-effectiveness ratios for additional scenarios

	AFB	AFB + NAAT	Increment
TB low scenario			
Costs	$2,105.43	$2,148.83	$43.39
Outcomes	1.394	1.396	0.002
ICER			$18,533
Perfect clinical judgment			
Costs	$2,016.20	$2,119.79	$103.59
Outcomes	1.397	1.397	0.0001
ICER			$724,423
TB high scenario			
Costs	$6,544.41	$6,692.36	$147.95
Outcomes	1.391	1.391	0.0001
ICER			$1,713,838

AFB = acid-fast bacilli; ICER = incremental cost-effectiveness ratio; NAAT = nucleic acid amplification test

NAAT is most cost-effective in the scenario in which all patients are managed as though they have a low clinical suspicion of TB. In this scenario, treatment decisions are driven by the results of AFB ± NAAT. The PASC protocol indicated that when AFB and NAAT results were discordant, treatment decisions would be based on NAAT, and so the introduction of NAAT in this scenario has the greatest ability to change patient management. NAAT is more sensitive than AFB, and this leads to improved management in patients with TB as there are fewer false-negative results. In patients without TB, change in patient management with NAAT occurs in two ways:

- in patients with an initial AFB (false)-positive result, NAAT may correctly identify no TB (i.e. NAAT may improve patient management), and decreases in the specificity of NAAT lead to the same treatment outcomes as if tested with AFB
- in patients with an initial AFB (true)-negative result, NAAT may falsely identify TB, and decreases in the specificity of NAAT lead to more false-positive results (i.e. NAAT leads to detrimental patient management).

Given that the pooled estimate of AFB specificity is high (98%), false-positive results are likely to be driven by the specificity of NAAT in AFB-negative results. As the pooled estimate for this parameter and that in AFB-positive results in low-incidence countries are also both high (99% and 97%, respectively), the net effect is a reduction in the number of false-positive results.

The cost-effectiveness of NAAT is least in the scenarios in which all patients (with TB, and with or without TB, respectively) are managed as though they have a high clinical suspicion of TB. In these scenarios treatment initiation decisions are based on clinical judgement, with the benefit of NAAT restricted to identifying drug resistance to initiate an appropriate treatment earlier. As the prevalence of MDR-TB in those with TB is approximately 2%, the benefits of NAAT are accrued in a very small proportion of the population tested (0.44%). As NAAT is associated with a net reduction in the number of false-positive results, the perfect clinical suspicion scenario, in which treatment initiation in true TB-negative patients is based on the results of AFB ± NAAT, is more cost-effective than the TB high scenario, in which all patients receive treatment.

Given that the TB mixed population is a combination of patients with high and low clinical suspicion of TB, it is unsurprising that the cost-effectiveness of NAAT lies between the respective estimates observed when all suspects are treated as though they have either a low or a high clinical suspicion of TB.

Sensitivity analyses

Sensitivity analyses for the base-case (TB mixed) scenario were conducted around a number of parameters included in the economic modelling (using 95%CI or plausible upper and lower limits). Analyses were presented in a tornado analysis (Figure 36). An additional sensitivity analysis was conducted using the test accuracy parameters for AFB and NAAT from all studies included in the clinical assessment (rather than those reported in studies conducted in a low-incidence setting only).

The tornado analysis indicates that the ICER is most sensitive to changes in the prevalence of TB in the tested population, the specificity of NAAT (ICERs exceeding $200,000 for the three NAAT specificity estimates) and the changes in the specificity of AFB.

There is considerable uncertainty in the prevalence of TB in the tested population (those with clinical signs and symptoms of TB). A best-guess estimate was applied in the model (22%) provided by the applicant. This estimate was similar to the prevalence of TB reported in the diagnostic accuracy of studies conducted in low-incidence countries (24%). A range of 10–30% was applied in the tornado analysis. Increasing the prevalence was observed to increase the cost-effectiveness of NAAT; therefore, conversely, decreasing the prevalence decreased the cost-effectiveness of NAAT, increasing the ICER to $967,000.

As previously described, NAAT may lead to an increase in false-positive results (and so false initiation of treatment) in those with an AFB-negative result based on the specificity of NAAT in AFB-negative patients, and the model is highly sensitive to this change. Any reduction in the specificity of this parameter (from 100%) increases the number of false-positive patients that receive detrimental treatment, leading to increases in cost and poorer quality of life. However, it should be noted that, as culture is an imperfect reference standard, some proportion of NAAT false-positive patients may truly have clinical disease (see ‘Comparison of NAAT and culture, using clinical diagnosis as a reference standard’).

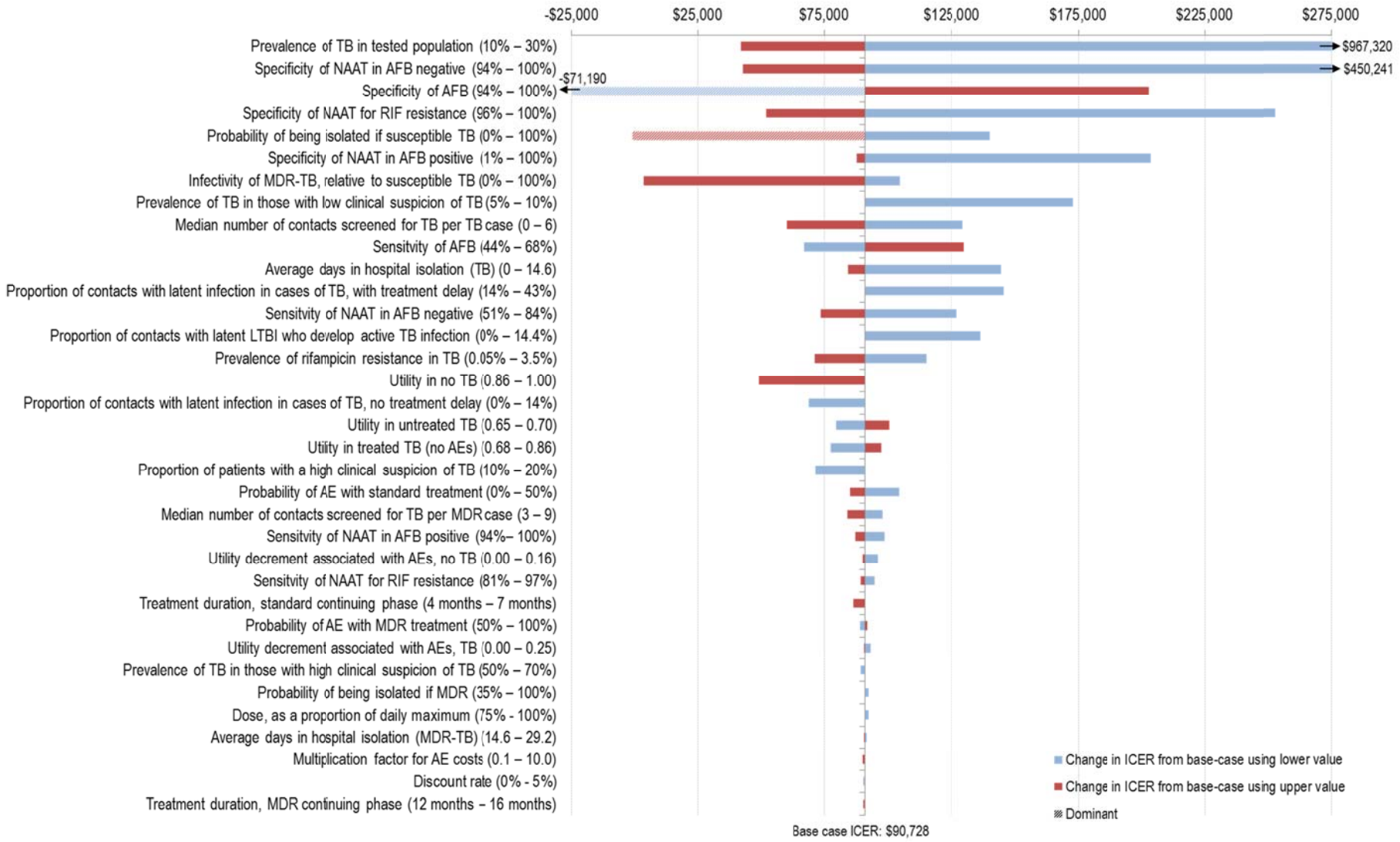

Figure 36 Tornado sensitivity analysis

AE = adverse event; AFB = acid-fast bacilli test; ICER = incremental cost-effectiveness ratio; LTBI = latent tuberculosis infection; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; RIF = rifampicin; TB = tuberculosis

As false-positive rifampicin resistance (in patients without TB and those with susceptible TB) is driven only by the specificity of NAAT for rifampicin resistance; reductions in this parameter increase the number of patients falsely treated with a more costly and more toxic treatment regimen.

The model is sensitive to the 95%CI of the specificity of NAAT in AFB-positive results, but this is more reflective of the imprecision of the pooled estimate (95%CI 1, 100) rather than the model being highly sensitive to changes in this variable alone.

Increases in the specificity of AFB (from 98% to 100%) increase the ICER substantially (to $203,000). As NAAT may correctly identify no TB in patients with an initial AFB (false)-positive result, reducing AFB false-positive results reduces the benefits of NAAT; in contrast, reducing the specificity of AFB (to 94%) increases the number of AFB false-positive results, substantially increasing the benefits of NAAT (dominant ICER).

Other variables that the ICER was seen to be sensitive to include:

- the probability of hospitalisation with susceptible TB—decreasing from 35% to 0% increases the ICER by 50% due to the net reduction in false-positive results associated with the introduction of NAAT
- the infectivity of MDR-TB relative to susceptible TB—increasing the infectivity coefficient from 30% to 100% (i.e. assuming the same relative infectivity as susceptible TB) decreases the ICER to $4,000, due to an increase in the number of MDR-TB transmissions, which disproportionally affects the comparator (as AFB cannot identify drug resistance)
- the median number of contacts screened per susceptible TB case—increasing the number from 3 to 6 decreased the ICER by one-third, due to the net reduction in false-negative results associated with the introduction of NAAT.

An additional sensitivity analysis was conducted using the results of AFB and NAAT by AFB status from all studies included in the clinical assessment, rather than those from a low-incidence setting alone (values presented in Table 46). The ICER is extremely sensitive to these changes and is predominantly driven by the combined decrease in the pooled estimates of the specificity of NAAT in AFB-negative results (from 99% to 94%) and the specificity of NAAT for rifampicin resistance (from 99% to 91%) (Table 64).

Table 64 Sensitivity analysis using test accuracy results of AFB, NAAT from all studies identified in the systematic review

	AFB	AFB + NAAT	Increment
Costs	$2,392.55	$3,045.78	$653.23
Outcomes	1.395	1.396	0.00002
ICER			$30,009,858

AFB = acid-fast bacilli; ICER = incremental cost-effectiveness ratio; NAAT = nucleic acid amplification test

Financial implications

A market-based approach is taken using MBS data to estimate the number of patients who accessed at least one of item of mycobacterial MC&S testing in 2009–13, and to project the expected number of patients who would be eligible for NAAT for TB and NTM (as requested) in 2015–19. One NAAT is assumed per eligible patient. As NAAT is not intended to replace current testing, the estimated net financial implication to the MBS is equal to the cost of the requested NAAT listings multiplied by the expected number of services.

Data sources used in the financial analysis

The sources for data used in the financial analysis are presented in Table 65.

Table 65 Data sources used in the financial analysis

Data	Source
Population eligible for NAAT	MBS data requested from the Department of Health relating to the number of patients who accessed at least one service from items 69324, 69325, 69327, 69328, 69330 or 69331 for the calendar years 2009–13. These data are projected to estimate the number of patients eligible for NAAT during 2015–19.
Proportion of patients eligible for NAAT suspected of TB	Applicant estimate of mycobacterial infections tested suspected of TB (50%). The inverse is the estimate of mycobacterial infections tested that are suspected of NTM.
Cost of NAAT for TB	As per 'Economic evaluation' ($130)
Cost of NAAT for NTM	Victorian Mycobacterium Reference Laboratory price per NAAT for suspected *M. ulcerans* infections ($50) or generic region *Mycobacterium* PCR ($120)
Proportion of patients bulk-billed	MBS data requested from the Department of Health regarding the proportion of patients who accessed at least one service from items 69324, 69325, 69327, 69328, 69330 or 69331 for the calendar years 2009–13 who were bulk-billed.

NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; TB = tuberculosis

Net financial implications to the MBS

The population eligible for NAAT is projected based on the number of patients who accessed mycobacterial MC&S testing (MBS items 69324, 69325, 69327, 69328, 69330 or 69331) each year during 2009–13 (Table 66). As these items are used to monitor the effectiveness of treatment and as this is not a proposed use of NAAT, the number of

patients rather than the number of services has been used. This patient pool accounts for the population suspected of TB (who can or cannot have an AFB) and the population suspected of NTM.

Table 66 Number of patients who accessed MC&S services, 2009–13

	2009	2010	2011	2012	2013
No. patients who accessed at least one MC&S service	28,188	27,853	30,213	32,857	34,302

MC&S = acid-fast bacilli microscopy, culture and sensitivity

A linear regression model fitted to the observed patient numbers ($R^2 = 0.92$) was projected to 2019 (Figure 37 and Table 67). This approach may overestimate the eligible population for NAAT, as patients tested who are suspected of *M. leprae* may be included (but would not be eligible for NAAT) and, as these tests are used to monitor treatment effectiveness, patients may receive testing across multiple years for the same infection. Further, as the current MBS items are not restricted to patients with clinical signs and symptoms of a mycobacterial infection, and as HESP member feedback has indicated that testing may be ordered as part of the initial work-up of a chronic obstructive pulmonary disease or some renal diseases, this approach may further overestimate the eligible population. This will be tested in a sensitivity analysis.

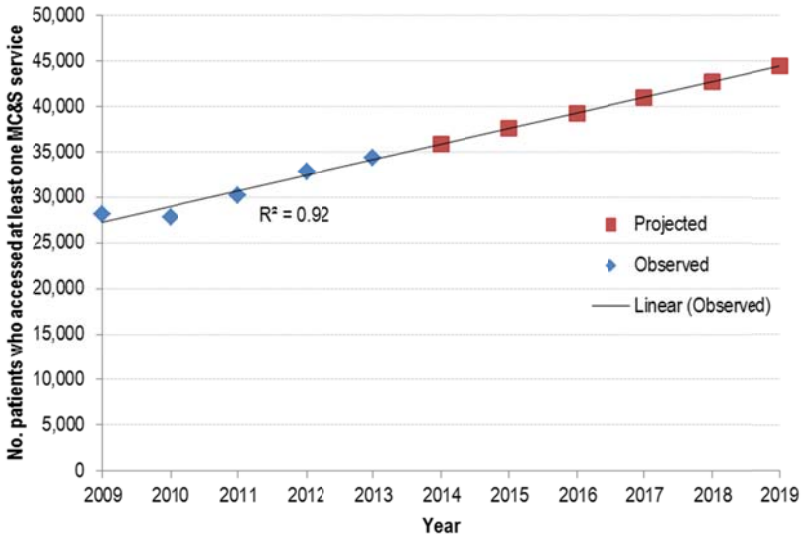

Figure 37 Number of patients who accessed MC&S services, observed 2009–13 and projected 2014–19
MC&S = acid-fast bacilli microscopy, culture and sensitivity

Furthermore, it is proposed that use of NAAT may earlier identify TB and MDR-TB, enabling faster treatment and decreasing the number of secondary transmissions. Neither the potential decrease in the rate of increasing cases due to reduced transmissions, nor the potential savings on treatment costs due to decreased transmissions, is captured in the financial estimates.

As the MBS items for MC&S do not distinguish between TB and NTM, and do not distinguish between those who do and do not have an AFB test, these projected patient numbers cannot be separated into the three proposed populations with any degree of confidence. As NAAT costs differ between TB and NTM, the applicant has estimated that approximately 50% of the patients currently tested for mycobacterial infections are suspected of TB, and so 50% are suspected of NTM.

Generally, one NAAT is assumed per eligible patient. However, this approach may underestimate the estimated number of tests (and so costs) in circumstances in which multiple mycobacteria are suspected. For example, TB may be initially suspected with a pulmonary infection (and therefore be tested using TB NAAT), and then may also be tested using NAAT for *M. kansasii* and/or MAC. It is unclear how often this situation would occur—the applicant has made an estimate of approximately 30% of patients initially suspected of TB. This is used in the estimation of the financial implications associated with NAAT for NTM and will be tested in sensitivity analyses.

NAAT for TB

The cost per TB NAAT is as used in the economic modelling ($130); the financial implications of a range of test costs are presented in Appendix J. Over the past 5 years average bulk-billing rates for the current MC&S items ranged from 59% to 62%; the midpoint (60.5%) will be used in the analysis. It is assumed that the provider does not charge above the MBS fee, and so the patient contribution, in those not bulk-billed, is 15% of the proposed NAAT fee.

Of all patients suspected of a mycobacterial infection, it is assumed that 50% are suspected of having TB (based on applicant advice). This estimate is tested in sensitivity analyses. Applying this proportion to the projected eligible population estimates, 18,800 patients are estimated to be eligible for TB NAAT in the first year, increasing to 22,200 in the fifth. The total MBS fees associated with the introduction of NAAT for TB increase from $2.4 million to $2.9 million over the 5-year period, of which $2.1 million in year 1 to $2.5 million in year 5 are paid by the MBS. Safety net effects to the MBS have not been considered in these calculations, as MBS data relating to the proportion of patients eligible for the safety net are not available. Patient contributions are estimated to increase from $145,000 to $171,000 over the 5 years. This may be an overestimate as, due to the contagious nature of TB, state TB services may waive all patient fees associated with the investigation of TB.

Table 67 Number of patients eligible and cost of NAAT for TB

	2015	2016	2017	2018	2019
Projected no. of patients eligible for NAAT	37,575	39,299	41,022	42,745	44,468
Population suspected of TB					
Proportion of patients suspected of TB	50%	50%	50%	50%	50%
Number of patients suspected of TB	18,788	19,650	20,511	21,373	22,234
Proposed NAAT fee:	$130.00	$130.00	$130.00	$130.00	$130.00
MBS benefit (85%)	$110.50	$110.50	$110.50	$110.50	$110.50
Patient contribution (15%)	$19.50	$19.50	$19.50	$19.50	$19.50
Proportion of patients bulk-billed	61%	61%	61%	61%	61%
MBS fees associated with TB listing:	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
MBS benefits payable (85%)	$2,076,074	$2,171,325	$2,266,466	$2,361,717	$2,456,857
Patient co-payments [a]	$144,715	$151,354	$157,986	$164,626	$171,257

[a] Only payable by patients who are not bulk-billed
NAAT = nucleic acid amplification test; TB = tuberculosis

NAAT for NTM

The cost per NTM NAAT is assumed as per the Victorian Mycobacterium Reference Laboratory cost for *M. ulcerans* ($50). Sensitivity analysis will be conducted using the generic region *Mycobacterium* PCR test cost ($120) (also from the Victorian reference laboratory). Bulk-billing rates are assumed as per NAAT for TB.

Patients eligible for NAAT for NTM include those initially suspected of an NTM infection (i.e. 50% of all patients suspected of a mycobacterial infection) and those initially suspected of TB, who may also receive testing for NTM (30% of initial TB suspects). In year 1, 24,400 patients are considered eligible for NAAT for NTM, increasing to 28,900 in year 5.

The total MBS fees associated with the introduction of NAAT for NTM increase from $1.2 million to $1.4 million over the 5-year period, of which $1.0 million in year 1 to $1.2 million in year 5 are paid by the MBS. Safety net effects to the MBS have not been considered in these calculations. Patient contributions are estimated to increase from $72,400 to $85,600 over the 5 years.

Table 68 Number of patients eligible and cost of NAAT for NTM

	2015	2016	2017	2018	2019
Projected no. of patients eligible for NAAT	37,575	39,299	41,022	42,745	44,468
Population suspected of NTM					
Proportion of patients suspected of NTM	50%	50%	50%	50%	50%
Number of patients suspected of NTM	18,788	19,650	20,511	21,373	22,234
Proportion of initial TB suspects tested	30%	30%	30%	30%	30%
Number of initial TB suspects tested	5,636	5,895	6,153	6,412	6,670

	2015	2016	2017	2018	2019
Total no. of patients tested for NTM	24,424	25,545	26,664	27,785	28,904
Proposed NAAT fee:	$50.00	$50.00	$50.00	$50.00	$50.00
MBS benefit (85%)	$42.50	$42.50	$42.50	$42.50	$42.50
Patient contribution (15%)	$7.50	$7.50	$7.50	$7.50	$7.50
Proportion of patients bulk-billed	61%	61%	61%	61%	61%
MBS fees associated with NTM listing:	$1,221,220	$1,277,250	$1,333,215	$1,389,245	$1,445,210
MBS benefits payable (85%)	$1,038,037	$1,085,663	$1,133,233	$1,180,858	$1,228,429
Patient co-payments [a]	$72,357	$75,677	$78,993	$82,313	$85,629

[a] Only payable by patients who are not bulk-billed
NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; TB = tuberculosis

The total MBS fees associated with the introduction of NAAT increase from $3.7 million to $4.3 million over the 5-year period, of which $3.1 million in year 1 to $3.7 million in year 5 are paid by the MBS. Patient contributions are estimated to increase from $217,000 to $257,000 over the 5 years (Table 69).

Table 69 Total cost of NAAT for requested listings

	2015	2016	2017	2018	2019
MBS fees associated with NAAT listings:	$3,663,660	$3,831,750	$3,999,645	$4,167,735	$4,335,630
MBS benefits payable (85%)	$3,114,111	$3,256,988	$3,399,698	$3,542,575	$3,685,286
Patient co-payments [a]	$217,072	$227,031	$236,979	$246,938	$256,886

[a] Only payable by patients who are not bulk-billed
NAAT = nucleic acid amplification test

As described, the approach used to estimate the population suspected of having a mycobacterial infection may overestimate the population eligible for NAAT, as current MBS item numbers do not restrict testing to those with the clinical signs and symptoms of a mycobacterial infection.

As the implications of the Medicare Safety Net were not included in the analysis, the MBS benefits payable could be underestimated.

Uncertainty scenarios

Uncertainties flagged around estimates used in the financial analysis were tested in sensitivity analyses (Table 70).

The analyses were most sensitive to increases in the NAAT cost (TB or NTM), increasing the total cost of NAAT by more than 60%. The assumption that 25% of all patients currently tested are not eligible for NAAT (e.g. those who do not have clinical signs and symptoms of mycobacterial infection) decreased the total cost of NAAT by the same proportion (25%). Changes to variables that increased the proportion of patients tested for TB (relative to

NTM), and increases in the proportion of patients initially suspected of TB tested for NTM, increased the total cost of NAAT slightly (16% and 3%, respectively).

Table 70 Sensitivity analyses

	2015	2016	2017	2018	2019
Base-case					
Total cost of NAAT for TB	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
Total cost of NAAT for NTM	$1,221,220	$1,277,250	$1,333,215	$1,389,245	$1,445,210
Total cost of NAAT	$3,663,660	$3,831,750	$3,999,645	$4,167,735	$4,335,630
Patients eligible for NAAT, 75% (base-case: 100%)					
Total cost of NAAT for TB	$1,831,830	$1,915,810	$1,999,920	$2,083,900	$2,167,880
Total cost of NAAT for NTM	$915,915	$957,905	$999,960	$1,041,950	$1,083,940
Total cost of NAAT	$2,747,745	$2,873,715	$2,999,880	$3,125,850	$3,251,820
Proportion of patients suspected of TB, 75% (base-case: 50%)					
Total cost of NAAT for TB	$3,663,530	$3,831,620	$3,999,710	$4,167,670	$4,335,630
Total cost of NAAT for NTM	$892,415	$933,360	$974,305	$1,015,185	$1,056,115
Total cost of NAAT	$4,555,945	$4,764,980	$4,974,015	$5,182,855	$5,391,745
Proportion of patients suspected of TB, 25% (base-case: 50%)					
Total cost of NAAT for TB	$1,221,220	$1,277,250	$1,333,280	$1,389,180	$1,445,210
Total cost of NAAT for NTM	$1,549,960	$1,621,075	$1,692,190	$1,763,240	$1,834,305
Total cost of NAAT	$2,771,180	$2,898,325	$3,025,470	$3,152,420	$3,279,515
Proportion of initial TB suspects tested for NTM, 20% (base-case: 30%)					
Total cost of NAAT for TB	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
Total cost of NAAT for NTM	$1,127,280	$1,179,000	$1,230,660	$1,282,380	$1,334,040
Total cost of NAAT	$3,569,720	$3,733,500	$3,897,090	$4,060,870	$4,224,460
Proportion of initial TB suspects tested for NTM, 40% (base-case: 30%)					
Total cost of NAAT for TB	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
Total cost of NAAT for NTM	$1,315,160	$1,375,500	$1,435,770	$1,496,110	$1,556,380
Total cost of NAAT	$3,757,600	$3,930,000	$4,102,200	$4,274,600	$4,446,800
Cost of NAAT for NTM, $120 (base-case: $50)					
Total cost of NAAT for TB	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
Total cost of NAAT for NTM	$2,930,928	$3,065,400	$3,199,716	$3,334,188	$3,468,504
Total cost of NAAT	$5,373,368	$5,619,900	$5,866,146	$6,112,678	$6,358,924

NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; TB = tuberculosis

Other Australian healthcare system costs

Costs to the state and territory health systems

There is some indication that NAAT is currently being used (though perhaps not routinely) in the diagnosis of mycobacterial infections. Advice from one state Mycobacterium Reference Laboratory indicated that these costs are currently covered by the states for public patients, and so listing of NAAT will shift these costs from state health budgets to the federal health budget.

Costs to the private health insurer and/or patient

Patient contributions associated with the proposed NAAT listings are estimated to increase from $217,000 to $257,000 over a 5-year period.

Some testing may currently be funded privately. It is assumed that some of these costs will shift to the federal health budget (subject to the fee and patient contribution).

Total Australian healthcare system costs

Given that some shifting of costs from state health budgets to the federal health budget is anticipated with the proposed listings, the net societal costs of NAAT may be lower than those presented in Table 69.

Conclusions

Is NAAT safe?

There were no studies on the safety of NAAT compared with current testing (AFB microscopy, tissue biopsy and/or culture). As NAAT is usually conducted on the same samples used for other testing, and there is no need for resampling, no AEs were expected.

To date, NAAT has been widely used without any safety concerns. However, more patients will receive a false-positive NAAT than a false-positive AFB result. Therefore, more patients will receive treatment for a disease they do not have and will possibly have an adverse reaction to the anti-TB drugs until clinical unresponsiveness is noted or culture results become available.

Is NAAT effective?

Direct evidence

Two studies were included that assessed the direct health impact of NAAT (Theron et al. 2014; Yoon et al. 2012). Both studies were conducted in a high prevalence setting and applicability to the Australian healthcare system is therefore questionable. A high-quality RCT reported no difference in morbidity outcomes at 2 and 6 months follow-up when NAAT and AFB microscopy were compared. However, a strong trend indicating fewer deaths in the NAAT group compared with the AFB microscopy group was observed at 2 months, but this trend was no longer apparent at 6 months. A historical control study of medium quality found no difference in the mortality rate at 2 months follow-up when comparing NAAT with no NAAT.

The authors of both studies suggested that high rates of treatment initiation based on empiric evidence in the no-NAAT groups probably underestimated the morbidity and/or mortality rate in the NAAT groups. Yoon et al. (2012) also suggested that sicker patients in the NAAT compared with the no-NAAT group contributed to this underestimation in their study.

Linked evidence

Is NAAT accurate in the diagnosis of MTB?

Comparison of NAAT and culture using clinical diagnosis as the reference standard

Culture is an imperfect reference standard as not all patients with a clinical diagnosis of TB (due to symptoms and response to anti-TB drugs) will be culture-positive. Meta-analysis to compare the sensitivity and specificity of culture and NAAT, using clinical diagnosis as a reference standard, indicated that 24% of patients clinically diagnosed with TB will have a false-negative culture result, compared with 14% having a false-negative NAAT. Thus, a large proportion of NAAT false-positive patients (i.e. NAAT-positive, culture-negative) would actually be clinically diagnosed as having TB. Therefore, NAAT is likely to be more effective at confirming the presence of an MTB infection than the meta-analysis using culture, as the reference standard would suggest.

AFB plus NAAT versus culture

Meta-analysis of studies investigating the diagnostic accuracy of AFB plus NAAT compared with culture showed that the overall pooled sensitivity (94%, 95%CI 91, 98) and specificity (88%, 95%CI 82, 92) values did not differ significantly to those for sputum and non-sputum specimens when analysed separately. Thus, 6% of patients will have a false-negative result, and 12% of patients (8% with sputum specimens and 17% with non-sputum specimens) will be false-positive.

The LR+ and LR– summary values for AFB microscopy plus NAAT compared with culture indicated that a negative AFB and NAAT result correctly identified most patients who were culture-negative and showed strong diagnostic evidence for confirmation of culture-positive TB. In sputum specimens, AFB plus NAAT correctly identified most patients as either culture-positive or culture-negative. As expected, the AUC for AFB microscopy plus NAAT, in both sputum and non-sputum specimens indicated that AFB plus NAAT performs well in predicting culture positivity.

NAAT versus culture

Meta-analysis of studies investigating the diagnostic accuracy of NAAT compared with culture showed that the pooled sensitivity (89%; 95%CI 85, 92) and specificity (94%; 95%CI 91, 96) values for all specimens did not differ significantly when sputum and non-sputum specimens were analysed separately. Consequently, 11% of patients (11% with sputum specimens and 9% with non-sputum specimens) will have false-negative results and 6% (5% with sputum specimens and 8% with non-sputum specimens) false-positive results when compared with culture results. The SROC curve showed some threshold effect, suggesting

that in-house NAAT is less specific than commercial NAAT when compared with culture, especially in countries with a high incidence of TB and when testing non-sputum specimens. However, both in-house NAATs and the commercial Xpert NAAT have diagnostic value for confirming or excluding culture-positive disease. Overall, patients with a positive NAAT result are likely to have culture-positive TB, whereas patients with a negative NAAT result are unlikely to be falsely negative.

In AFB-positive specimens the overall pooled sensitivity (99%; 95%CI 96, 100) and specificity (78%; 95%CI 53, 92) values of NAAT compared with culture did not differ significantly between sputum and non-sputum specimens, but the CIs for specificity were very wide. In contrast, in AFB-negative specimens the pooled sensitivity and specificity values differed between sputum (sensitivity = 67%; 95%CI 45, 84, and specificity = 96%; 95%CI 90, 99) and non-sputum (sensitivity = 86%; 95%CI 78, 91, and specificity = 86%; 95%CI 78, 91) specimens, but the difference did not quite reach statistical significance.

The summary LR values showed that both in-house NAATs and the commercial Xpert NAAT have diagnostic value in confirming or excluding culture-positive disease. Overall, the ability of NAAT to correctly diagnose the presence or absence of TB in patients when compared with culture suggested that patients with a positive NAAT result most likely actually have culture-positive TB. Conversely, patients with a negative NAAT result were more likely not to have culture-positive TB than to be falsely negative.

In the context of interpreting NAAT results in conjunction with AFB findings, when patients are AFB-positive a negative NAAT result could confidently rule out culture-positive MTB being detected in that patient, but a positive NAAT result did not eliminate the possibility of AFB-positive patients not having a detectable MTB infection (i.e. being culture-negative). The reduced certainty in interpreting a positive NAAT result is due to culture being an imperfect reference standard, which likely resulted in misclassification of many of the 22% false-positive results seen for NAAT when compared with culture in AFB-positive specimens.

In patients with AFB-negative specimens a positive NAAT result is likely to correctly confirm the presence of culture-positive MTB. However, interpretation of a negative NAAT result is dependent on the type of specimen tested. In patients with AFB-negative sputum a negative NAAT indicated that the patient may not be culture-positive but it cannot be ruled out. In patients with AFB-negative non-sputum specimens a negative NAAT result provided no additional useful information. This is likely due to the paucibacillary nature of AFB-negative specimens. It should be noted that if few bacilli are present in the specimen, the possibility of a false-negative result would increase for all three tests.

Meta-analysis of studies investigating the diagnostic accuracy of NAAT compared with culture in HIV-positive and -negative patients showed that there was no difference in diagnostic accuracy among the three tests. However, as HIV-positive patients with pulmonary TB commonly produce AFB-negative sputum specimens (de Albuquerque et al. 2014; Scherer et al. 2011), the difficulty associated with diagnosis of TB in HIV-positive patients is related to the reduced sensitivity of NAAT in AFB-negative compared with AFB-positive specimens.

AFB versus culture

Meta-analysis of studies investigating the diagnostic accuracy of AFB compared with culture showed that AFB microscopy was significantly more sensitive in identifying MTB in sputum (71%; 95%CI 59, 81) compared with non-sputum (46%; 95%CI 37, 55) specimens. Overall, 38% of all patients (29% with sputum specimens and 54% with non-sputum specimens) will have a false-negative AFB microscopy result, compared with only 2% with a false-positive result. The pooled specificity for AFB microscopy was 98% (95%CI 97, 99) for all specimens and was similar when sputum and non-sputum specimens were analysed separately. These results were confirmed by the SROC curve, which showed that there was a threshold effect based on specimen type, with sensitivity being higher in sputum specimens than non-sputum specimens.

For specific specimen types the pooled sensitivity for AFB microscopy compared with culture varied from 46% in urine to 62% in FNAs of lymph nodes. However, for CSF the pooled sensitivity was only 11%. Thus, AFB microscopy is not a useful tool for diagnosis of TB in CSF specimens. The pooled specificity was at least 94% in all specimen types.

The summary LR+ and LR– values for the ability of AFB microscopy to correctly diagnose the presence or absence of TB in patients when compared with culture suggest that patients with a positive AFB test result are most likely to actually have TB than not. However, patients with a negative test result may or may not have TB, indicating that AFB microscopy provides no useful information in these patients.

Comparison of AFB, NAAT and AFB plus NAAT using culture as the reference standard in HIV-positive patients

The pooled sensitivity and specificity values for AFB microscopy and/or NAAT compared with culture in HIV-positive and -negative populations were compared with those for all included studies, which largely consisted of patients in whom their HIV status was unknown. No differences between the pooled values for the three population groups were observed,

indicating that HIV status does not affect the performance of either AFB microscopy or NAAT.

HIV-positive patients with pulmonary TB commonly produce AFB-negative sputum specimens (de Albuquerque et al. 2014; Scherer et al. 2011). Thus, the difficulty associated with diagnosis of TB in HIV-positive patients is related to the reduced sensitivity of NAAT compared with culture in AFB-negative specimens, as discussed above.

NAAT versus culture-based DST

Meta-analysis of studies investigating the diagnostic accuracy of NAAT compared with culture-based DST showed that NAAT is both highly sensitive (93%; 95%CI 85, 97) and highly specific (98%; 95%CI 96, 99) compared with DST in identifying rifampicin-resistant MTB. Thus, NAAT could be used to inform appropriate treatment decisions, possibly avoiding side effects such as hepatitis from inappropriate use of rifampicin.

However, there was insufficient evidence to determine if NAAT could be used as a surrogate for the detection of MDR-MTB by detecting mutations in the *rpoB* gene that confer rifampicin resistance. Only 2 studies reported data for this comparison, with vastly different point estimates and enormous 95%CIs for sensitivity, and no conclusion could be reached.

Does it change patient management?

Fourteen studies reported results on time to TB diagnosis or anti-TB treatment after NAAT compared with AFB microscopy or culture, with 8 of these studies conducted in countries with a relatively high TB prevalence. It was shown that time to diagnosis was shorter with Xpert compared with liquid and solid culture, and similar to AFB microscopy. Median time to treatment was also decreased with the use of Xpert compared with other methods of diagnosis, especially culture. The proportion of TB patients diagnosed and initiating treatment on the day of presentation was higher when NAAT was used in addition to AFB microscopy. Furthermore, laboratory turnaround time was significantly shorter for Xpert and AFB microscopy, compared with culture. The median time for rifampicin-resistance detection was 1 day (IQR 0–1) for Xpert, compared with 20 days (IQR 10–26) for line probe assay and 106 days (IQR 30–124) for phenotypic susceptibility testing. Other time-related management results were reported in three studies conducted in low-prevalence countries, all reporting a decrease in time to identification of MTB infection when NAAT was used.

Thus, not surprisingly, all studies were in agreement that the use of NAAT resulted in a quicker diagnosis of patients with TB, especially in those who were AFB-negative. Predictably, this also resulted in earlier treatment in NAAT-positive patients.

Other changes in management were also reported. A historical control study of low quality and a retrospective cohort study of medium quality reported that the median duration of unnecessary and/or over-treatment of TB was shorter in patients when NAAT was used to guide treatment decisions compared with those when NAAT was not available. The retrospective cohort study also reported that culture-negative NAAT-negative patients had significantly fewer average days on outpatient medications compared with other groups.

There were conflicting data on the likely impact of NAAT in the clinical setting. A retrospective cohort study of low quality and a high risk of bias conducted in the UK (medium TB incidence; 15/100,000 people) reported that NAAT resulted in a change in management in 39% of patients. The authors concluded that there were significant clinical benefits from the use of NAAT in low-prevalence settings, with additional benefits when used with AFB-positive specimens (Taegtmeyer et al. 2008).

On the other hand, a lack of change in management with no discontinuation of treatment after a negative Xpert result was reported in two cohort studies of medium quality, one retrospective and conducted in Saudi Arabia (medium TB incidence; 15/100,000 people) and the other conducted in Canada (low TB incidence; 4.6/100,000 people). Omrani et al. (2014) concluded that physicians who are highly experienced in the diagnosis and treatment of TB underused the Xpert NAAT and it had only a limited impact on their decisions related to starting or stopping anti-TB therapy.

Thus, while there is no doubt that NAAT results would be available much faster than culture results and that patients could be started on anti-TB treatment much sooner, there was conflicting data on the likely impact of NAAT in the clinical setting.

Does change in management improve patient outcomes?

What health impact does early versus delayed treatment of TB have on the individual and their contacts?

Two prospective cohort studies, conducted in countries with a low incidence of TB (Italy and the USA), reported that a delay in time to diagnosis, defined as the period from onset of any TB symptoms to the initiation of anti-TB treatment, was significantly associated with an increased risk of transmission of infection among contacts. Although these results are not surprising, they reinforce the belief that quicker diagnosis of TB is of great benefit in reducing the spread of TB to the close contacts of infected individuals.

The results of a retrospective cohort study of poor quality, conducted in New Zealand, indicated that the time between development of symptoms and diagnosis was not

significantly associated with the odds of achieving a favourable treatment outcome. As 'favourable treatment outcome' was poorly defined in this study, this result may simply reflect the treatment completion rate, which appears to be unrelated to any treatment delays.

To what extent does treating patients who have a rifampicin-resistant MTB infection with alternative treatments result in better health outcomes for the patient and their contacts?

No studies were identified that met all the PICO criteria. However, three cohort studies (two retrospective) of medium quality provided some evidence regarding the research question. These studies suggested that patients who received a rifampicin-containing Category II treatment before receiving the DST results had poorer treatment outcomes than those who did not.

What are the AEs associated with unnecessary antibiotic treatment?

All TB patients are at risk of adverse health events associated with first-line treatments, irrespective of their appropriateness. For example, the development of hepatitis was associated with the use of rifampicin and pyrazinamide, either separately or in combination (Table 32). Hepatitis occurred more often in older patients, and skin rashes were more common in patients who were female, older, HIV-infected or from Asia. Patients with chronic renal failure tended to have a higher incidence of AEs from anti-TB regimens, in particular neuropsychiatric events. Also, AEs occurred less often in children than in adults. However, data providing the evidence on AEs was non-comparative and came primarily from countries with high or medium incidences of TB, where patients may also have been sicker with co-morbidities or had poorer nutrition, limiting their relevance in an Australian setting. Nevertheless, two SRs, one of medium quality and one of poor quality, found that some but not all AEs as a consequence of patients with active TB receiving inappropriate antibiotic treatment (due to MTB resistance) may be avoided with appropriate treatment, to which the MTB strain is sensitive.

More importantly, from a public health perspective, one SR of good quality found that patients who received inappropriate treatment, as defined by the WHO treatment guidelines for MDR-TB (WHO 2008), had a 27-fold increased risk of developing drug resistance than if they received an appropriate treatment regimen. Thus, earlier identification of drug-resistant strains via NAAT could be beneficial in preventing inappropriate treatment and the further spread of MDR-TB.

Overall conclusion with respect to comparative effectiveness

Comparison of AFB, NAAT, and AFB plus NAAT using culture as the reference standard showed that AFB plus NAAT (the testing strategy proposed in the application) has the highest false-positive rate, at 12%, with NAAT alone at 6% and AFB alone at 2%. A false-positive result means that a patient will receive treatment for a short time (until clinical unresponsiveness is noted or culture results are available) for a disease they do not have. However, as culture is an imperfect reference standard, a large proportion of these false-positive patients may actually have clinical disease. AFB microscopy alone has the highest false-negative rate, at 38%, with NAAT alone and AFB plus NAAT being much lower at 11% and 6%, respectively. The consequences of a false-negative result are much more severe, as the patient may remain untreated for a longer time period and could potentially spread the disease to more individuals in the community.

The results of the meta-analyses presented in this report suggest that NAAT would be a useful addition to AFB microscopy and culture in the diagnosis of both pulmonary and extrapulmonary TB. Patients with a positive AFB test result or a positive NAAT are most likely to have culture-positive TB, and it becomes almost certain if both tests are positive. No useful information can be obtained directly from a negative AFB result, as these patients may or may not have TB. A negative NAAT result should be interpreted with reference to the AFB result—a negative NAAT result in a patient who was AFB-positive almost completely eliminates the likelihood of being MTB culture-positive. Conversely, a negative NAAT result in a patient who was AFB-negative does not eliminate the possibility of having culture-positive disease.

The use of NAAT enables quicker diagnosis and treatment of patients with TB, especially in those who are NAAT-positive and AFB-negative. It also reduces the duration of unnecessary and/or over-treatment for TB, especially in those patients who are NAAT-negative and AFB-positive.

The accuracy of NAAT compared with culture-based DST indicates that NAAT can accurately identify patients with rifampicin-resistant MTB. Thus, NAAT could be used to inform the best type of antibacterial treatment of TB patients. This would help avoid side effects such as hepatitis from inappropriate use of rifampicin, and earlier appropriate treatment for rifampicin resistance would also reduce the risk of developing MDR-TB.

Is NAAT accurate in the diagnosis of NTM?

Culture is an imperfect reference standard, and meta-analysis of studies investigating the diagnostic accuracy of NAAT, AFB microscopy and culture using a clinical reference standard

suggested that most patients who were NAAT-positive and culture-negative may have had clinical disease. Overall, NAAT appears to be able to identify a larger proportion of patients with an NTM infection than either AFB microscopy or culture. Additionally, the diagnostic accuracy results for NTM-NAAT and MAC-NAAT should be viewed with caution due to the small number of studies included and the wide 95%CIs for many of the analyses.

NAAT to detect NTM could be separated into three distinct categories: NAAT to detect NTMs in general (NTM-NAAT), NAAT to specifically detect *M. avian* complex (MAC) strains (MAC-NAAT), and NAAT to detect *M. ulcerans* in patients suspected of having Buruli ulcer. The pooled sensitivity and specificity values for NTM-NAAT compared with culture indicated that 24% of culture-positive patients would have false-negative results, but only 2% of culture-negative patients would have false-positive results. For MAC-NAAT compared with culture, 41% of culture-positive patients would have false-negative results and no culture-negative patient would have false-positive results. The summary LR+ and LR– values for the ability of MAC-NAAT to correctly diagnose the presence or absence of NTM infections in patients when compared with culture suggest that patients with a positive NAAT result are likely to actually have an infection, but patients with a negative NAAT result may or may not have an NTM infection. Conversely, patients with a negative NTM-NAAT are more likely to not have an NTM infection than to have one, but whether patients with a positive result actually have an infection is less certain. The SROC curve shows some threshold effect, suggesting that MAC-NAAT may be more sensitive and less specific than NTM-NAAT when compared with culture. Nevertheless, the AUC indicated that both NTM-NAAT and MAC-NAAT perform well in predicting culture positivity.

AFB microscopy was not very useful in identifying patients who do not have NTM infections when compared with culture. The pooled sensitivity values indicated that 53% of culture-positive patients and 69% of patients with a positive clinical diagnosis received a false-negative AFB result. The LR scattergram indicated that patients with a positive AFB test result were most likely to actually have an NTM infection, but patients with a negative test result may or may not have an NTM infection (AFB microscopy provides no useful information in these patients). The SROC AUC also indicated that AFB microscopy performed only moderately well in predicting culture positivity.

Is NAAT cost-effective?

The base-case ICER for NAAT (the TB mixed scenario) is $90,728/QALY. The addition of NAAT leads to more patients initially receiving the correct treatment, due to improved sensitivity of NAAT in conjunction with AFB and the ability to identify MDR-TB. The incremental cost of

NAAT is driven predominantly by the cost of testing, offset by reduced TB transmissions and hospitalisation costs. The incremental QALY gain is driven by the shift of TB patients from being initially untreated (or having standard treatment in the case of MDR-TB) to receiving correct treatment.

The cost-effectiveness of NAAT is affected by the extent of use of clinical judgement in initial treatment decisions. In the extreme scenario, in which clinical judgment is not exerted (i.e. treatment initiation decisions are based on the results of testing), NAAT is most cost-effective due to improved sensitivity in conjunction with AFB, thereby reducing the number of patients who would have been untreated on the basis of AFB results alone. However, in the scenarios in which clinical judgement perfectly identifies TB or in which clinical judgment is used as the basis to treat all patients, the benefits of NAAT are restricted to identifying rifampicin resistance, and so are accrued in a very small proportion of the population tested (2% of 22% = 0.44%).

Substantial uncertainty surrounds a number of variables included in the economic modelling, in particular the prevalence of TB in the tested population. The ICER is most sensitive to changes in this variable; for example, decreasing the estimated prevalence in the tested population from 22% to 10% increases the ICER to $967,000.

The ICER is also sensitive to decreases in the specificity of NAAT, particularly in AFB-negative results (e.g. using the lower limit of the 95%CI increases the ICER to $450,000) and for rifampicin resistance (e.g. using the lower limit of the 95%CI increases the ICER to $253,000). Any decrease in these specificities (from 100%) increases the number of false-positive patients that receive poorly tolerated treatment, leading to increases in cost and poorer quality of life. However, as culture is an imperfect reference standard for diagnosis of TB, some proportion of NAAT false-positive patients may truly have clinical disease, and so the uncertainty in the ICER associated with reductions in the specificity in AFB-negative results may be an overestimate.

Costing

Given the uncertainties in estimating the eligible population, the financial implications of introducing NAAT are uncertain. However, as NAAT is proposed to be used as an add-on test, net costs to the MBS are implied. Estimates presented in the assessment ($3.7 million to $4.3 million over the 5-year period) are likely to represent the upper limits of proposed use, as all assumptions regarding the eligible population are likely to be overestimated. The

financial implications are most sensitive to changes in the cost per test. While benefits associated with reduced transmissions may be expected, these have not been quantified.

As NAAT is currently being used (the extent of which is uncertain), some shifting of costs from the states to the federal health budget is anticipated, and so the net financial implications to the Australian healthcare system are likely to be less than the net cost of introducing NAAT to the MBS.

Appendix A Health Expert Standing Panel and Assessment Group

Health Expert Standing Panel (HESP)

Member	Expertise or affiliation
Jim Black	Associate Professor, Nossal Institute for Global Health, Melbourne School of Population and Global Health

Assessment group

AHTA, University of Adelaide, South Australia

Name	Position
Judy Morona	Senior Research Officer
Arlene Vogan	Health Economist
Sharon Kessels	Research Officer
Debra Gum	Senior Research Officer
Joanne Milverton	Research Officer
Jacci Parsons	Team Leader (Medical HTA)
Skye Newton	Team Leader (Medical HTA)
Camille Schubert	Senior Health Economist
Tracy Merlin	Managing Director

Noted conflicts of interest

There were no conflicts of interest.

Appendix B Search strategies

HTA websites

INTERNATIONAL	
International Network of Agencies for Health Technology Assessment	http://www.inahta.org/
AUSTRALIA	
Australian Safety and Efficacy Register of New Interventional Procedures – Surgical (ASERNIP-S)	http://www.surgeons.org/for-health-professionals/audits-and-surgical-research/asernip-s/
Centre for Clinical Effectiveness, Monash University	http://www.monashhealth.org/page/Health_Professionals/CCE/
Centre for Health Economics, Monash University	http://www.buseco.monash.edu.au/centres/che/
AUSTRIA	
Institute of Technology Assessment / HTA unit	http://www.oeaw.ac.at/ita
CANADA	
Institut National d'Excellence en Santé et en Services Sociaux (INESSS)	http://www.inesss.qc.ca/en/publications/publications/
Alberta Heritage Foundation for Medical Research (AHFMR)	http://www.ahfmr.ab.ca/publications.html
Alberta Institute of Health Economics	http://www.ihe.ca/
The Canadian Agency for Drugs And Technologies in Health (CADTH)	http://www.cadth.ca/index.php/en/
The Canadian Association for Health Services and Policy Research (CAHSPR)	http://www.cahspr.ca/
Centre for Health Economics and Policy Analysis (CHEPA), McMaster University	http://www.chepa.org
Health Utilities Index (HUI), McMaster University	http://www.chepa.mcmaster.ca/hug/index.htm
Centre for Health Services and Policy Research (CHSPR), University of British Columbia	
Institute for Clinical and Evaluative Studies (ICES)	http://www.ices.on.ca
Saskatchewan Health Quality Council (Canada)	http://www.hqc.sk.ca
DENMARK	
Danish National Institute Of Public Health	http://www.si-folkesundhed.dk/?lang=en
FINLAND	
Finnish National Institute for Health and Welfare	http://www.thl.fi/en/web/thlfi-en/
FRANCE	
L'Agence Nationale d'Accréditation et d'Evaluation en Santé (ANAES)	http://www.anaes.fr/
GERMANY	
German Institute for Medical Documentation and Information (DIMDI) / HTA	http://www.dimdi.de/static/en/index.html
Institute for Quality and Efficiency in Health Care (IQWiG)	http://www.iqwig.de
THE NETHERLANDS	
Health Council of the Netherlands Gezondheidsraad	http://www.gezondheidsraad.nl/en/
Institute for Medical Technology Assessment (Netherlands)	http://www.imta.nl/

NEW ZEALAND	http://www.otago.ac.nz/christchurch/research/nzhta/
New Zealand Health Technology Assessment (NZHTA)	
NORWAY	http://www.kunnskapssenteret.no
Norwegian Knowledge Centre for the Health Services	
SPAIN	
Agencia de Evaluación de Tecnologias Sanitarias, Instituto de Salud "Carlos III"I/Health Technology Assessment Agency (AETS)	http://www.isciii.es/
Andalusian Agency for Health Technology Assessment (Spain)	http://www.juntadeandalucia.es/
Catalan Agency for Health Technology Assessment (CAHTA)	http://www.gencat.cat
SWEDEN	
Center for Medical Technology Assessment, Linköping University	http://www.cmt.liu.se/?l=en&sc=true
Swedish Council on Technology Assessment in Health Care (SBU)	http://www.sbu.se/en/
SWITZERLAND	
Swiss Network on Health Technology Assessment (SNHTA)	http://www.snhta.ch/
UNITED KINGDOM	
National institute for Health Research, Health Technology Assessment Programme	http://www.hta.ac.uk/
NHS Quality Improvement Scotland	http://www.nhshealthquality.org/
National Institute for Clinical Excellence (NICE)	http://www.nice.org.uk/
The European International Network on New and Changing Health Technologies	http://www.euroscan.bham.ac.uk/
University of York NHS Centre for Reviews and Dissemination (NHS CRD)	http://www.york.ac.uk/inst/crd/
UNITED STATES	
Agency for Healthcare Research and Quality (AHRQ)	http://www.ahrq.gov/clinic/techix.htm
Harvard School of Public Health	http://www.hsph.harvard.edu/
Institute for Clinical and Economic Review (ICER)	http://www.icer-review.org/
Institute for Clinical Systems Improvement (ICSI)	http://www.icsi.org
Minnesota Department of Health (US)	http://www.health.state.mn.us/
National Information Centre of Health Services Research and Health Care Technology (US)	http://www.nlm.nih.gov/nichsr/nichsr.html
Oregon Health Resources Commission (US)	http://www.oregon.gov/oha/OHPR/HRC/Pages/index.aspx
Office of Health Technology Assessment Archive (US)	http://ota.fas.org/
U.S. Blue Cross/ Blue Shield Association Technology Evaluation Center (Tec)	http://www.bcbs.com/blueresources/tec/
Veteran's Affairs Research and Development Technology Assessment Program (US)	http://www.research.va.gov/default.cfm

Additional sources of literature

Source	Location
Internet	
NHMRC- National Health and Medical Research Council (Australia)	http://www.nhmrc.gov.au/
US Department of Health and Human Services (reports and publications)	http://www.hhs.gov/
New York Academy of Medicine Grey Literature Report	http://www.greylit.org/
Trip database	http://www.tripdatabase.com
Current Controlled Trials metaRegister	http://controlled-trials.com/
National Library of Medicine Health Services/Technology Assessment Text	http://text.nlm.nih.gov/
U.K. National Research Register	http://www.nihr.ac.uk/Pages/NRRArchive.aspx
Google Scholar	http://scholar.google.com/
Australian and New Zealand Clinical Trials Registry	www.anzctr.org.au
World Health Organization	http://www.who.int/en/
Pearling	
All included articles will have their reference lists searched for additional relevant source material	
Guidelines search (last step linked evidence)	
Guidelines International Network (G-I-N)	http://www.g-i-n.net/
NHMRC Clinical Guidelines Portal	http://www.clinicalguidelines.gov.au

Additional databases searched for economic evaluations

Electronic database	Time period
Database of Abstracts of Reviews of Effects or Reviews of Effects (DARE)	to 19 May 2014
Health Technology Assessment database	to 19 May 2014
NHS Economic Evaluation Database (NHS EED)	to 19 May 2014

Appendix C Diagnostic accuracy 2x2 data from included studies

Table 71 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in mixed specimens

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Maurya et al. (2011b) India	Level III-1 Low risk of bias	BACTEC culture	N=328 extrapulmonary specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	23 140 140	14 63 71	128 11 11	163 114 106	20/479 specimens had contaminated cultures and were excluded from analysis
			n=37	AFB +ve: IS*6110* PCR	23	6	0	8	
			n=291	AFB –ve: IS*6110* PCR	117	57	11	106	
Fan et al. (2014) China	Level III-1 Low risk of bias	MGIT liquid culture	N=200 respiratory samples	AFB –ve: SAT-TB PCR	57	6	4	133	None reported
Kim et al. (2008) Korea	Level III-2 Low risk of bias	Ogawa media culture	N=2,973 specimens	AFB (FL + ZN) IS*6110* PCR AFB + NAAT	118 142 148	2 56 56	79 55 49	2,774 2,720 2,720	None reported
Deshmukh et al. (2013) India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=463 specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	96 165 165	15 75 75	88 19 19	264 204 204	3/466 cultures grew NTM and were excluded from analysis
			n=111	AFB +ve: IS*6110* PCR	96	15	0	0	
			n=352	AFB –ve: IS*6110* PCR	69	60	19	204	
			N= 436 non-sputum	AFB (ZN) IS*6110* PCR AFB + NAAT	91 152 152	14 72 72	79 18 18	152 194 194	
			n=105	AFB +ve: IS*6110* PCR	91	14	0	0	
			n=331	AFB –ve: IS*6110* PCR	61	58	18	194	
			N=423 extrapulmonary	AFB (ZN) IS*6110* PCR AFB + NAAT	89 147 147	14 70 70	76 18 18	244 188 188	
			n=103	AFB +ve: IS*6110* PCR	89	14	0	0	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
			n=320	AFB –ve: IS*6110* PCR	58	56	18	188	
Ben Kahla et al. (2011) Tunisia	Level III-2 Low risk of bias	L-J culture	N=316 specimens	All: AFB (FL) IS*6110* PCR AFB + NAAT	50 48 51	10 6 14	5 7 4	251 249 247	23/333 sputum samples were contaminated and not included in analysis
			n=60	AFB +ve: IS*6110* PCR	47	2	3	8	
			n=256	AFB –ve: IS*6110* PCR	1	4	4	247	
Therese, Jayanthi & Madhavan (2005) India	Level III-2 Low risk of bias	L-J culture	N=280 extrapulmonary clinical samples	AFB (ZN) IS*6110* PCR AFB + NAAT	8 13 13	1 115 116	6 1 1	265 151 150	None reported
			n=9	AFB +ve: IS*6110* PCR	8	0	0	1	
			n=271	AFB –ve: IS*6110* PCR	5	115	1	150	
Jiang et al. (2012) China	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=235 mixed samples	AFB (ZN) 16S qRT-PCR AFB + NAAT	28 34 34	32 25 49	8 2 2	167 174 150	None reported
			n=60	AFB +ve: 16S qRT-PCR	28	8	0	24	
			n=175	AFB –ve: 16S qRT-PCR	6	17	2	150	
			N=28 extrapulmonary	AFB (ZN) 16S qRT-PCR AFB + NAAT	4 6 6	5 4 6	2 0 0	17 18 16	
			n=9	AFB +ve: 16S qRT-PCR	4	3	0	2	
			n=19	AFB –ve: 16S qRT-PCR	2	1	0	16	
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=178 extrapulmonary specimens	AFB (ZN) IS*6110* PCR	6 4	4 44	0 2	168 128	None reported
Gholoobi et al. (2014) Iran	Level III-2 Low risk of bias	L-J culture	N=30 mixed specimens	AFB (ZN) 16S-23S PCR *rpoB* PCR IS*6110* PCR	9 8 5 7	3 4 0 0	3 4 7 5	15 15 18 18	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Ani et al. (2009) Nigeria	Level III-2 Low risk of bias	L-J slope culture	N=40 mixed specimens from children	AFB –ve: IS*6110* PCR	4	18	0	18	None reported
			N=30 non-sputum samples		0	12	0	18	
Halse et al. (2010) USA	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=1,309 specimens	AFB (ZN)	225	42	45	997	7/1,316 specimens had no PCR result Inconclusive PCR: (not reproducible) 5 respiratory specimens 2 non-respiratory Indeterminate PCR: (PCR inhibition) 3 respiratory specimens
				IS*6110 rpoB* qPCR	253	1	17	1,038	
				AFB + NAAT	254	43	16	996	
			n=267	AFB +ve: IS*6110 rpoB* qPCR	224	0	1	42	
			n=1,042	AFB –ve: IS*6110 rpoB* qPCR	29	1	16	996	
			N=1,196 pulmonary	AFB (ZN)	211	37	38	910	
				IS*6110 rpoB* qPCR	235	1	14	946	
				AFB + NAAT	236	38	13	909	
			n=248	AFB +ve: IS*6110 rpoB* qPCR	210	0	1	37	
			n=948	AFB –ve: IS*6110 rpoB* qPCR	25	1	13	909	
			N=113 extrapulmonary	AFB (ZN)	14	5	7	87	
				IS*6110 rpoB* qPCR	18	0	3	92	
				AFB + NAAT	18	5	3	87	
			n=19	AFB +ve: IS*6110 rpoB* qPCR	14	0	0	5	
			n=94	AFB –ve: IS*6110 rpoB* qPCR	4	0	3	87	
Drouillon et al. (2009) France and Italy	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=607 specimens	AFB (ZN + FL)	61	0	70	476	1/633 specimen was culture contaminated, 12/633 were PCR-2 inhibited, 13/633 were positive NTM and excluded from analysis
				qRT-PCR	115	13	16	463	
			N=548 pulmonary	AFB (ZN + FL)	60	0	59	429	
				qRT-PCR	105	11	14	418	
			N=59 extrapulmonary	AFB (ZN + FL)	1	0	11	47	
				qRT-PCR	10	2	2	45	
Shukla et al. (2011) India	Level III-2 Some risk of bias	L-J culture	N=140 specimens	AFB (ZN)	38	2	10	90	Not reported
				IS*6110* nPCR	46	58	2	34	
				AFB + NAAT	48	60	0	32	
			n=40	AFB +ve: IS*6110* nPCR	36	0	2	2	
			n=100	AFB –ve: IS*6110* nPCR	10	58	0	32	
			N=86 pulmonary	AFB (ZN)	38	2	8	38	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
				IS*6110* nPCR	44	20	2	20	
				AFB + NAAT	46	22	0	18	
			n=40	AFB +ve: IS*6110* nPCR	36	0	2	2	
			n=46	AFB –ve: IS*6110* nPCR	8	20	0	18	
			N=54 extrapulmonary	AFB –ve: IS*6110* nPCR	2	38	0	14	
Drouillon et al. (2007) France	Level III-2 Some risk of bias	MGIT or Coletsos slants	N=168 pulmonary specimens	AFB	11	0	21	136	11/179 cultures were contaminated and excluded from analysis
				IS*6110* qPCR	20	9	12	127	
Sharma et al. (2012) India	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=80 extrapulmonary specimens	AFB (ZN)	1	0	2	77	None reported
				M-PCR	3	63	0	14	
				AFB + NAAT	3	63	0	14	
			n=79	AFB –ve M-PCR	2	63	0	14	

AFB = acid-fast bacilli; FL = fluorescent; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; nPCR = nested PCR; PCR = polymerase chain reaction; qPCR = quantitative (real-time) PCR; qRT-PCR = quantitative (real-time) reverse transcription PCR; ZN = Ziehl-Neelsen

Table 72 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in mixed specimens

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Park et al. (2013) Korea	Level III-1 Low risk of bias	Liquid and/or solid culture	N=320 pulmonary specimens	AFB	13	13	10	284	None reported
				Xpert	19	6	4	291	
				AFB + NAAT	22	19	1	278	
			n=26	AFB +ve: Xpert	10	0	3	13	
			n=294	AFB –ve: Xpert	9	6	1	278	
Tortoli et al. (2012) Italy	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=1,413 extrapulmonary samples	AFB (FL)	98	12	140	1163	61/1,493 specimens grew NTM 17/1,493 had indeterminate Xpert results and were excluded from analysis
				Xpert	188	32	50	1143	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bates et al. (2013) Zambia	Level III-2 Low risk of bias	MGIT culture	N=930 mixed pulmonary specimens from children	AFB (FL) Xpert	15 42	43 16	30 7	842 865	None reported
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=239 specimens	AFB (FL) Xpert AFB + NAAT	41 58 59	1 0 1	20 4 3	177 177 176	None reported
			n=43	AFB +ve: Xpert	41	0	1	1	
			n=196	AFB –ve: Xpert	17	0	3	176	
			N=183 non-sputum	AFB (FL) Xpert AFB + NAAT	10 21 22	1 0 1	13 3 2	159 159 158	
			n=12	AFB +ve: Xpert	10	0	1	1	
			n=171	AFB –ve: Xpert	11	0	2	158	
			N=67 extrapulmonary	AFB (FL) Xpert AFB + NAAT	6 16 17	0 0 0	11 2 1	50 49 49	
			n=7	AFB +ve: Xpert	6	0	1	0	
			n=60	AFB –ve: Xpert	10	0	1	49	
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=180 specimens	AFB (FL) Xpert AFB + NAAT	15 29 29	2 2 4	16 2 2	147 147 145	None reported
			n=17	AFB +ve: Xpert	15	0	0	2	
			n=163	AFB –ve: Xpert	14	2	2	145	
			N=162 non-sputum	AFB (FL) Xpert AFB + NAAT	11 24 24	2 2 4	15 2 2	134 134 132	
			n=13	AFB +ve: Xpert	11	0	0	2	
			n=149	AFB –ve: Xpert	13	2	2	132	
			N=89 extrapulmonary	AFB (FL) Xpert AFB + NAAT	4 12 12	1 2 3	10 2 2	74 73 72	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
			n=5	AFB +ve: Xpert	4	0	0	1	
			n=84	AFB –ve: Xpert	8	2	2	72	
Balcells et al. (2012) Chile	Level III-2 Low risk of bias	Solid L-J and liquid media culture	N=160 HIV+ sputum or mouthwash specimens	HIV+ AFB (ZN)	8	2	4	146	Repeated Xpert MTB/RIF assays were performed for patients who had discordant results
				Xpert	11	1	1	147	
Teo et al. (2011) Singapore	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=153 specimens	AFB (FL)	47	11	30	65	9/162 respiratory specimens gave invalid GeneXpert results
				Xpert	70	8	7	68	
				AFB + NAAT	70	14	7	62	
			n=58	AFB +ve: Xpert	47	5	0	6	
			n=95	AFB –ve: Xpert	23	3	7	62	
			N= 122 pulmonary	: AFB (FL)	43	9	19	51	
				Xpert	56	5	6	55	
				AFB + NAAT	56	11	6	49	
			n=52	AFB +ve: Xpert	43	3	0	6	
			n=70	AFB –ve: Xpert	13	2	6	49	
			N=31 extrapulmonary	AFB (FL)	4	2	11	14	
				Xpert	14	3	1	13	
				AFB + NAAT	14	3	1	13	
			n=6	AFB +ve: Xpert	4	2	0	0	
			n=25	AFB –ve: Xpert	10	1	1	13	
Ioannidis et al. (2011) Greece	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=92 AFB -ve mixed specimens	AFB –ve: Xpert	19	5	3	65	12/105 cultures were contaminated 1/105 had invalid Xpert result
Deggim et al. (2013) Switzerland	Level III-2 Low risk of bias	MGIT 960 liquid and Middlebrook 7H11 culture	N=77 mixed specimens	AFB (FL)	14	5	4	54	2/79 had invalid Xpert results
				Xpert	15	2	3	57	
				AFB + NAAT	15	7	3	52	
			n=19	AFB +ve Xpert	14	0	0	5	
			n=58	AFB –ve Xpert	1	2	3	52	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Zeka, Tasbakan & Cavusoglu (2011) Turkey	Level III-2 Some risk of bias	MB/BacT liquid and/or L-J culture	N=429 mixed specimens	AFB (FL) Xpert AFB + NAAT	31 71 71	1 6 7	58 18 18	339 334 333	None reported
			n=32	AFB +ve: Xpert	31	0	0	1	
			n=397	AFB –ve: Xpert	40	6	18	333	
Marlowe et al. (2011) USA	Level III-2 Some risk of bias	Culture	N=216 pulmonary specimens	AFB Xpert AFB + NAAT	87 116 118	39 4 39	43 14 12	47 82 47	1/217 bronchial specimen was found to be PCR inhibitory and was excluded from analysis
			n=126	AFB +ve: Xpert	85	4	2	35	
			n=90	AFB –ve: Xpert	31	0	12	47	
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=147 AFB –ve extrapulmonary specimens	AFB –ve: Xpert	63	0	45	39	2/149 specimens had Xpert indeterminate results

AFB = acid-fast bacilli; FL = fluorescent; HIV = human immunodeficiency virus; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; TB = tuberculosis; Xpert = GeneXpert MTB/RIF NAAT; ZN = Ziehl-Neelsen

Table 73 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in sputum

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Davis et al. (2009) Uganda	Level II Low risk of bias	L-J Middlebrook and MGIT cultures	N=100 sputum samples	AFB (ZN) *secA1* PCR AFB + NAAT	55 62 63	8 6 11	8 1 0	29 31 26	27/127 AFB microscopy results not recorded and excluded from analysis
			n=63	AFB +ve: *secA1* PCR	54	3	1	5	
			n=37	AFB –ve *secA1* PCR	8	3	0	26	
Mashta et al. (2011) India	Level III-1 Low risk of bias	L-J liquid medium culture	N=463 sputum samples	AFB (MB) IS*6110* -*devR* PCR AFB + NAAT	148 69 157	80 71 115	44 123 39	191 200 152	Contaminated samples (4%) were identified and patients were called to collect a second sample
			n=228	AFB +ve IS*6110* -devR PCR	60	32	88	48	
			n=235	AFB –ve IS*6110* -devR PCR	9	35	39	152	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Nakiyingi et al. (2012) Uganda	Level III-1 Some risk of bias	L-J liquid medium culture	N=181 patients with AFB –ve sputum samples	AFB –ve IS*6110* PCR	48	75	16	42	5/205 cultures were contaminated 19/205 PCR results were unavailable
Ekrami et al. (2011) Iran	Level III-1 Some risk of bias	L-J solid medium culture	N=152 sputum samples	AFB (ZN) IS*6110* PCR IS*6110* nPCR)	90 78 94	6 8 6	16 28 12	40 38 40	None reported
Chakravorty et al. (2006) India	Level III-2 Low risk of bias	L-J solid medium culture	N=506 sputum samples from 506 patients	USP AFB (ZN) *devR* PCR IS*6110* PCR	273 269 272	39 52 65	0 4 1	194 181 168	None reported
Michelon et al. (2011) Brazil	Level III-2 Low risk of bias	Culture	N=469 sputum specimens N=295 induced sputum specimens N=174 spontaneous sputum specimens	AFB IS*6110* PCR AFB + NAAT AFB IS*6110* PCR AFB + NAAT AFB IS*6110* PCR AFB + NAAT	62 65 75 55 51 60 7 14 15	7 7 12 5 5 10 2 2 2	14 10 1 6 9 1 8 1 0	386 387 381 229 230 224 157 157 157	4/476 (3 induced and 1 spontaneous) specimens were PCR inhibitory 3/479 (induced) gave indeterminate results
Jiang et al. (2012) China	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=207 sputum samples n=51 n=156	AFB (ZN) 16S qRT-PCR AFB + NAAT AFB +ve: 16S qRT-PCR AFB –ve: 16S qRT-PCR	24 28 28 24 4	27 21 43 5 16	6 2 2 0 2	150 156 134 22 134	None reported
Suzuki et al. (2006) Japan	Level III-2 Low risk of bias	MGIT culture	N=138 sputum specimens n=55 n=83	: AFB *dnaJ* PCR-ICA AFB + NAAT AFB +ve: *dnaJ* PCR-ICA AFB –ve: *dnaJ* PCR-ICA	53 41 57 39 1	2 5 5 2 3	20 32 16 14 19	63 60 60 0 60	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
El Khechine et al. (2009) France	Level III-2 Low risk of bias	BACTEC 9000 liquid culture	N=134 sputum samples N=134 stool samples	AFB (ZN) IS*6110* qPCR AFB + NAAT	13 18 18	2 10 10	7 0 0	112 106 106	None reported
George, Mony & Kenneth (2011) India	Level III-2 Low risk of bias	MGIT and/or L-J medium culture	N=71 sputum samples n=33 n=38	AFB (FL) LAMP AFB + NAAT AFB +ve: LAMP AFB –ve: LAMP	32 31 32 29 2	1 2 3 2 0	7 8 7 1 7	31 30 29 1 29	7/78 samples showed contamination for both L-J and MGIT cultures and were omitted from analysis
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=27 sputum specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	5 13 13	1 3 3	9 1 1	12 10 10	None reported
Ani et al. (2009) Nigeria	Level III-2 Low risk of bias	L-J slope culture	N=10 sputum specimens from children	AFB –ve IS*6110* PCR	4	0	6	0	None reported
Chakravorty et al. (2005) India	Level III-2 Some risk of bias	L-J solid medium culture	N=571 samples from 571 patients	direct AFB (ZN) USP AFB (ZN) IS*6110* PCR	322 225 325	21 18 70	6 103 3	222 225 173	None reported
Santos et al. (2006) Brazil	Level III-2 Some risk of bias	L-J medium culture	N=214 sputum samples n=46 n=168	AFB (direct) AFB (conc.) IS*6110* PCR AFB (conc.) + NAAT AFB +ve: IS*6110* PCR AFB –ve: IS*6110* PCR	14 17 38 38 17 21	1 4 24 26 2 22	30 27 6 6 5 1	169 166 146 144 22 124	4/218 samples were excluded due to contamination of culture tubes
Lee, Chen & Peng (2009) Taiwan	Level III-2 Some risk of bias	Culture (not specified)	N=150 sputum samples	AFB LAMP	30 32	9 7	4 2	107 109	None reported
de Albuquerque et al. (2014) Brazil	Level III-2 Some risk of bias	L-J solid medium and 7H9 broth culture	N=140 sputum specimens from 140 HIV+ patients	HIV+ AFB (ZN) IS*6110* qPCR	37 41	0 1	10 6	93 92	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Marchi et al. (2008) Brazil	Level III-2 Some risk of bias	L-J-MTBAC culture	N=117 sputum specimens	AFB (ZN) IS*6110* PCR	3 3	0 0	3 3	111 111	None reported
Ereqat et al. (2011) Palestine	Level III-2 Some risk of bias	Culture	N=95 samples from 84 patients n=13 n=82	AFB (ZN) IS*6110* PCR AFB + NAAT AFB +ve: IS*6110* PCR AFB –ve: IS*6110* PCR	7 10 10 7 3	6 20 20 6 14	4 1 1 0 1	78 64 64 0 64	None reported
Shukla et al. (2011) India	Level III-2 Some risk of bias	L-J culture	N=40 AFB +ve sputum specimens	AFB +ve: IS*6110* nPCR	36	0	2	2	None reported
Gomez et al. (2011) Southern Texas (USA) and Mexico	Level III-2 Some risk of bias	MGIT liquid medium and L-J solid medium culture	N=150 patients n=101 n=49	AFB *RD1* qPCR IS*1081* qPCR IS*6110* qPCR AFB + NAAT (IS*6110* qPCR) AFB +ve: IS*6110* qPCR AFB –ve: IS*6110* qPCR	99 102 102 104 107 96 8	2 0 0 0 2 0 0	8 5 5 3 0 3 0	41 43 43 43 41 2 41	32 had contaminated MGIT
Haldar et al. (2007) India	Level III-2 Some risk of bias	7H9 liquid media culture	N=148 sputum samples	Direct AFB (ZN) USP AFB (ZN) *devR* FL-PCR *devR* Gel-PCR IS*6110* FL-PCR IS*6110* Gel-PCR	24 85 111 109 110 102	2 2 2 5 4 3	96 35 9 11 10 18	26 26 26 23 24 25	None reported

AFB = acid-fast bacilli; FL = fluorescent; HIV = human immunodeficiency virus; LAMP = loop-mediated isothermal amplification; L-J = Lowenstein-Jensen; MB = methylene blue; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; nPCR = nested PCR; PCR = polymerase chain reaction; PCR-ICA = PCR-immunochromatographic assay; qPCR = quantitative (real-time) PCR; qRT-PCR = quantitative (real-time) reverse transcription PCR; USP = universal sample processing; ZN = Ziehl-Neelsen

Table 74 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in sputum

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Rachow et al. (2011) Tanzania	Level II Low risk of bias	MGIT and/or L-J media culture	N=249 sputum samples	AFB (ZN) Xpert AFB + NAAT	51 61 62	0 8 8	18 8 7	180 172 172	43/292 were Xpert indeterminate and excluded from final analysis
			n=51	AFB +ve: Xpert	50	0	1	0	
			n=198	AFB –ve: Xpert	11	8	7	172	
Walusimbi et al. (2013) Uganda	Level III-1 Low risk of bias	MGIT and/or L-J culture	N=369 AFB –ve HIV+ sputum specimens with valid results	HIV+ AFB –ve Xpert	21	16	22	310	57/430 specimens had contaminated cultures 19/430 had invalid Xpert results
Carriquiry et al. (2012) Peru	Level III-1 Low risk of bias	MGIT and/or L-J media culture	N=131 HIV+ patients	HIV+ AFB (ZN) Xpert AFB + NAAT	31 44 44	3 2 4	14 1 1	83 84 82	1 culture was contaminated 2 Xpert results were invalid and repeated
			n=34	AFB +ve: Xpert	31	1	0	2	
			n=97	ABF –ve: Xpert	13	1	1	82	
Nicol et al. (2011) South Africa	Level III-2 Low risk of bias	MGIT culture	N=452 children with at least one induced sputum sample	AFB (FL) Xpert	27 52	0 6	43 18	382 376	None reported
Zar et al. (2013) South Africa	Level III-2 Low risk of bias	MGIT culture	N=384 children with at least one induced sputum sample	AFB (FL) Xpert	4 13	1 3	26 17	353 351	None reported
Kurbatova et al. (2013) Russia	Level III-2 Low risk of bias	MGIT and/or L-J media culture	N=228 specimens	AFB (direct) AFB (FL) Xpert (direct) Xpert (conc.)	65 93 102 103	3 12 17 16	44 16 5 6	124 114 104 111	2/238 culture results were missing 8/238 had Indeterminate Xpert results
Bates et al. (2013) Zambia	Level III-2 Low risk of bias	MGIT culture	N=142 sputum specimens from children	AFB (FL) Xpert	3 9	7 2	7 1	125 130	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Helb et al. (2010) Vietnam	Level III-2 Low risk of bias	MGIT and/or L-J media culture	N=107 patients	AFB	29	0	53	25	None reported
				Xpert	67	0	15	25	
				AFB + NAAT	67	0	15	25	
			n=29	AFB +ve: Xpert	29	0	0	0	
			n=78	AFB –ve: Xpert	38	0	15	25	
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=56 sputum specimens	AFB (FL)	31	0	7	18	None reported
				Xpert	37	0	1	18	
				AFB + NAAT	37	0	1	18	
			n=31	AFB +ve: Xpert	31	0	0	0	
			n=25	AFB –ve: Xpert	6	0	1	18	
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=18 sputum specimens	AFB (FL)	4	0	1	13	None reported
				Xpert	5	0	0	13	
				AFB + NAAT	5	0	0	13	
			n=14	AFB –ve: Xpert	1	0	0	13	
Hanrahan et al. (2014) South Africa	Level III-2 Some risk of bias	MGIT media culture	N=2,082 patients	AFB	186	10	186	1,472	None reported
				Xpert	299	47	107	1,638	
Theron et al. (2012) South Africa	Level III-2 Some risk of bias	MGIT medium culture	N=480 sputum samples	AFB (FL)	102	3	47	328	None reported
				Xpert	115	15	34	316	
				AFB + NAAT	122	17	27	314	
			n=286	HIV –ve: AFB (FL)	65	2	19	200	
				Xpert	68	10	16	193	
				AFB + NAAT	72	10	12	192	
			n=130	HIV +ve: AFB (FL)	26	1	24	79	
				Xpert	35	4	15	76	
				AFB + NAAT	37	5	13	75	
Sohn et al. (2014) Canada	Level III-2 Some risk of bias	Liquid culture	N=435 sputum samples	AFB (FL)	7	4	17	407	1/436 invalid Xpert result
				Xpert	11	1	13	410	
				AFB + NAAT	12	5	12	406	
			n=11	AFB +ve: Xpert	6	0	1	4	
			n=424	AFB –ve: Xpert	5	1	12	405	

AFB = acid-fast bacilli; FL = fluorescent; HIV = human immunodeficiency virus; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; Xpert = GeneXpert MTB/RIF NAAT; ZN = Ziehl-Neelsen

Table 75 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in bronchoalveolar lavage, bronchial aspirates and washings

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Min et al. (2010) Korea	Level III-2 Low risk of bias	Ogawa medium culture	N=136 bronchial aspirates	AFB stain (ZN) *senX3-regX3* qPCR	20 39	1 1	45 26	70 70	None reported
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=13 BAL specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	2 5 5	0 2 2	3 0 0	8 6 6	None reported
Kibiki et al. (2007) Tanzania	Level III-2 Some risk of bias	L-J culture	N=116 BAL samples	HIV+ AFB (ZN) IS*6110* PCR	16 27	1 42	12 1	87 46	4/120 specimens were contaminated (1 AFB +ve and 3 AFB –ve)

AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; PCR = polymerase chain reaction; qPCR = quantitative (real-time) PCR; ZN = Ziehl-Neelsen

Table 76 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in bronchoalveolar lavage, bronchial aspirates and washings

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Theron et al. (2013) South Africa	Level III-2 Low risk of bias	MGIT medium culture	N=154 BAL specimens	AFB (FL)	15	1	12	126	1/156 specimens had contaminated cultures 1/156 had Xpert error and was excluded from the analysis
				Xpert	25	5	2	122	
			n=135	AFB –ve: Xpert	9	4	2	120	
			n=84	HIV– AFB (FL)	7	0	5	72	
				Xpert	12	3	0	69	
				HIV– AFB –ve Xpert	5	3	0	69	
			n=46	HIV+ AFB (FL)	4	1	3	37	
				Xpert	6	2	2	34	
				HIV+ AFB –ve Xpert	1	1	7	34	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=116 BAL specimens n=111	AFB (FL) Xpert AFB –ve: Xpert	4 5 1	1 0 0	2 1 1	109 110 109	None reported
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=40 specimens 31 bronchial aspirates 9 BAL n=35	AFB (FL) Xpert AFB + NAAT AFB –ve: Xpert	4 7 7 3	1 0 1 0	3 0 0 0	32 33 32 32	None reported

AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; FL = fluorescent; HIV = human immunodeficiency virus; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF NAAT

Table 77 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in gastric aspirates

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bates et al. (2013) Zambia	Level III-2 Low risk of bias	MGIT culture	N=788 gastric aspirates from children	AFB (FL) Xpert	12 33	23 5	36 15	717 735	None reported
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=33 gastric aspirates n=30	AFB (FL) Xpert AFB + NAAT AFB –ve: Xpert	3 5 5 2	0 0 0 0	2 0 0 0	28 28 28 28	None reported
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=6 gastric aspirates	AFB –ve: Xpert	2	0	1	3	2/8 specimens had an Xpert indeterminate result

AFB = acid-fast bacilli; FL = fluorescent; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF NAAT

Table 78 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in stools

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
El Khechine et al. (2009) France	Level III-2 Low risk of bias	L-J for stools	N=134 stool samples	AFB (ZN) IS*6110* qPCR AFB + NAAT	9 13 13	0 11 11	4 0 0	121 110 110	None reported

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; NAAT = nucleic acid amplification test; qPCR = quantitative (real-time) polymerase chain reaction; ZN = Ziehl-Neelsen

Table 79 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in cerebrospinal fluid

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bhigjee et al. (2007) South Africa	Level III-2 Low risk of bias	7H11 medium culture and MGIT culture	N=126 CSF specimens from 68 patients	AFB –ve: IS*6110-MPB64* PCR qPCR	18 18	9 16	2 2	97 90	None reported
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=60 CSF specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	2 10 10	0 19 19	12 4 4	46 27 27	None reported
Desai et al. (2006) India	Level III-2 Low risk of bias	L-J culture	N=30 CSF specimens	AFB (ZN) IS*6110* PCR	1 8	0 12	19 0	22 10	None reported
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=25 CSF specimens	AFB –ve: IS*6110* PCR	0	9	0	16	None reported
Ani et al. (2009) Nigeria	Level III-2 Low risk of bias	L-J slope culture	N=5 CSF specimens from children	AFB –ve IS*6110* PCR	0	0	0	5	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Therese, Jayanthi & Madhavan (2005) India	Level III-2 Some risk of bias	L-J culture	N=120 CSF specimens n=119	AFB (ZN) IS*6110* PCR AFB + NAAT AFB –ve: IS*6110* PCR	1 1 1 0	0 37 37 37	0 0 0 0	119 82 82 82	None reported
Baveja et al. (2009) India	Level III-2 Some risk of bias	L-J and BACTEC culture	N=100 CSF specimens from children	AFB (ZN) *MPB64* PCR	2 22	0 0	20 0	78 78	None reported
Shukla et al. (2011) India	Level III-2 Some risk of bias	L-J culture	N=16 CSF specimens	AFB –ve: IS*6110* nPCR	0	12	0	4	None reported

AFB = acid-fast bacilli; CSF = cerebrospinal fluid; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; nPCR = nested PCR; PCR = polymerase chain reaction; qPCR = quantitative (real-time) PCR; ZN = Ziehl-Neelsen

Table 80 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in cerebrospinal fluid

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=15 CSF specimens	AFB –ve: Xpert	1	0	0	14	None reported
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=14 CSF specimens	AFB –ve: Xpert	0	0	0	14	None reported
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=14 CSF specimens	AFB –ve: Xpert	2	0	0	12	None reported

AFB = acid-fast bacilli; CSF = cerebrospinal fluid; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; Xpert = GeneXpert MTB/RIF NAAT

Table 81 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in body fluids

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Maurya et al. (2011a) India	Level III-1 Low risk of bias	BACTEC culture	N=102 pleural effusions	AFB (ZN) IS*6110* PCR AFB + NAAT	15 45 45	2 17 18	32 2 2	53 38 37	None reported
			n=17	AFB +ve IS*6110* PCR	15	1	0	1	
			n=85	AFB –ve IS*6110* PCR	30	16	2	37	
Singh et al. (2006) India	Level III-1 Low risk of bias	L-J culture	N=85 bone-marrow aspirates	AFB –ve *MPB64* PCR	1	18	0	66	None reported
Bhanu et al. (2005) India	Level III-1 Low risk of bias	L-J culture	N=16 endometrial aspirates	AFB (ZN) *mpt64* PCR	0 0	1 1	0 8	15 7	1 negative PCR included in analysis was found to be inhibitory
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=119 CSF specimens 59 ascetic fluid, 54 pleural fluid, 6 synovial fluid	AFB (ZN) IS*6110* PCR	1 0	0 28	0 1	118 90	None reported
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=52 body fluid specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	3 12 12	0 7 7	13 4 4	36 29 29	None reported
Ani et al. (2009) Nigeria	Level III-2 Low risk of bias	L-J slope culture	N=25 fluid specimens from children 11 gastric wash 5 ascitic fluid 9 pleural effusions	AFB –ve IS*6110* PCR	12	0	0	13	None reported
Therese, Jayanthi & Madhavan (2005) India	Level III-2 Some risk of bias	L-J culture	N=107 specimens 104 peritoneal and 3 pericardial	AFB (ZN) IS*6110* PCR AFB + NAAT	1 2 2	0 64 64	2 1 1	104 40 40	None reported
			n=106	AFB –ve: IS*6110* PCR	1	64	1	40	

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bhanothu, Theophilus & Rozati (2014) India	Level III-2 Some risk of bias	L-J culture	N=11 pelvic fluids	AFB (ZN) *TCR4* PCR AFB + NAAT	7 11 11	0 0 0	0 0 0	4 0 0	None reported

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; M-PCR = multiplex PCR; NAAT = nucleic acid amplification test; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 82 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in body fluids

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=22 specimens 13 pleural fluids 3 pericardial fluids 2 synovial fluids 4 abdominal aspirates	AFB –ve: Xpert	3	0	0	19	None reported
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=20 specimens 5 joint fluid 12 pleural fluid 3 peritoneal fluid	AFB –ve: Xpert	4	0	2	14	None reported
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=44 specimens 31 pleural fluid 7 joint fluid 3 ascitic fluid 3 pericardial fluid	AFB –ve: Xpert	13	0	22	9	None reported

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; Xpert = GeneXpert MTB/RIF NAAT

Table 83 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in fine-needle aspirates

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=12 FNA specimens	AFB (ZN) IS*6110* PCR	2 1	3 3	0 1	7 7	None reported
Derese et al. (2012) Ethiopia	Level III-2 Some risk of bias	L-J culture	N=134 FNAs from lymph nodes	n=101 AFB (ZN) n=124 IS*6110* nPCR	8 21	5 8	27 29	61 66	None reported
Pahwa et al. (2005) India	Level III-2 Some risk of bias	L-J medium culture	N=55 FNAs	AFB (ZN + FL) *MPB64* PCR	14 17	5 5	5 1	31 32	None reported
Mittal et al. (2011) India	Level III-2 Some risk of bias	L-J culture	N=50 lymph node FNAs	AFB (ZN) IS*6110* PCR AFB + NAAT	22 27 30	2 3 3	8 3 1	18 17 16	None reported
Therese, Jayanthi & Madhavan (2005) India	Level III-2 Some risk of bias	L-J culture	N=44 lymph node FNA specimens n=38	AFB (ZN) IS*6110* PCR AFB + NAAT AFB –ve: IS*6110* PCR	5 9 9 4	1 10 11 10	4 0 0 0	34 25 24 24	None reported

AFB = acid-fast bacilli; FL = fluorescent; FNA = fine-needle aspirate; L-J = Lowenstein-Jensen; NAAT = nucleic acid amplification test; nPCR = nested PCRPCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 84 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in fine-needle aspirates

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Van Rie et al. (2013b) South Africa	Level III-2 Low risk of bias	MGIT culture	N=344 FNA specimens	HIV+ AFB (FL) Xpert AFB + NAAT	59 139 140	5 23 23	87 10 9	188 172 172	8/373 cultures were contaminated or non-interpretable 4/373 were NTM

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests		True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
										5/373 had invalid Xpert results and were excluded from analysis
Ablanedo-Terrazas et al. (2014) Mexico	Level III-2 Low risk of bias	MGIT 960 liquid culture	N=68 lymph node FNAs	HIV+	AFB (ZN) Xpert AFB + NAAT	12 15 15	4 0 4	3 0 0	49 53 49	No invalid Xpert results were obtained
Ligthelm et al. (2011) South Africa	Level III-2 Low risk of bias	MGIT 960 liquid culture	N=48 lymph node FNAs		AFB (ZN) AFB (FL) Xpert AFB + NAAT	12 22 28 29	0 1 3 2	17 7 1 0	19 18 16 17	No invalid Xpert results were obtained
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=23 lymph nodes n=20	 AFB –ve:	AFB (FL) Xpert AFB + NAAT Xpert	3 6 6 3	0 0 0 0	3 0 0 0	17 17 17 17	None reported
Biadglegne et al. (2014) Ethiopia and Germany	Level III-2 Some risk of bias	L-J and Gottsacker culture	N=213 FNAs from lymph nodes n=12 n=201	 AFB +ve AFB –ve	AFB (FL) Xpert AFB + NAAT Xpert Xpert	11 29 29 11 18	1 56 56 1 55	20 2 2 0 2	181 126 126 0 126	11/231 cultures were contaminated 8/231 Xpert results were indeterminate and excluded from analysis
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=38 lymph nodes	AFB –ve:	Xpert	24	0	10	4	None reported
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=5 FNAs		AFB (FL) Xpert	2 2	1 0	0 0	2 3	None reported

AFB = acid-fast bacilli; FL = fluorescent; FNA = fine-needle aspirate; HIV = human immunodeficiency virus; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; Xpert = GeneXpert MTB/RIF NAAT; ZN = Ziehl-Neelsen

Table 85 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in abscesses/pus

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=94 pus specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	43 53 53	5 15 15	12 2 2	34 24 24	None reported
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=7 pus specimens	AFB (ZN) IS*6110* PCR	1 1	0 2	0 0	6 4	None reported

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 86 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in abscesses/pus

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=10 abscess aspirates n=8	AFB (FL) Xpert AFB –ve: Xpert	2 3 1	0 0 0	1 0 0	7 7 7	None reported
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=19 abscess aspirates	AFB –ve: Xpert	13	0	4	2	None reported

AFB = acid-fast bacilli; FL = fluorescent; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; Xpert = GeneXpert MTB/RIF NAAT

Table 87 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in urine

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=53 urine specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	4 15 15	2 9 9	16 5 5	31 24 24	None reported
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2 Low risk of bias	L-J culture	N=8 urine specimens	AFB (ZN) IS*6110* PCR	2 2	1 2	0 0	5 4	Not reported
Khosravi et al. (2010) Iran	Level III-2 Some risk of bias	L-J culture	N=200 urine samples	AFB (ZN) IS*6110* nPCR	4 10	0 0	6 0	190 190	None reported
Ghaleb, Afifi & El-Gohary (2013) Egypt	Level III-2 Some risk of bias	BACTEC 12B and/or L-J culture	N=100 urine samples n=99	AFB (ZN) IS*6110* PCR AFB + NAAT AFB –ve IS*6110* PCR	1 3 3 2	0 3 3 3	0 0 0 0	99 94 94 94	None reported
Khan, Cheema & Khan (2013) Egypt	Level III-2 Some risk of bias	L-J culture	N=50 urine samples	AFB (ZN) PCR	11 16	2 2	8 3	29 29	None reported

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; nPCR = nested PCR; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 88 Diagnostic accuracy of in-house NAAT compared with AFB microscopy and culture in tissue biopsies

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bhanu et al. (2005) India	Level III-1 Low risk of bias	L-J culture	N=14 endometrial biopsies	AFB (ZN) *mpt64* PCR	1 1	0 7	0 0	13 6	3 negative PCR included in analysis were found to be inhibitory

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Deshmukh et al. 2013 India	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=164 tissue specimens	AFB (ZN) IS*6110* PCR AFB + NAAT	37 57 57	7 3 3	23 3 3	97 84 84	None reported
Sharma et al. (2013) India	Level III-2 Low risk of bias	L-J culture	N=50 endoscopic ileocaecal biopsies	AFB (ZN) M-PCR	1 2	1 0	0 29	47 19	None reported
Shukla et al. (2011) India	Level III-2 Some risk of bias	L-J culture	N=38 endometrial biopsies	AFB –ve: IS*6110* nPCR	2	26	0	10	None reported
Bhanothu, Theophilus & Rozati (2014) India	Level III-2 Some risk of bias	L-J culture	N=191 endometrial or ovarian biopsies n=37 n=154	AFB (ZN) *TCR4* PCR AFB + NAAT AFB +ve: TCR4 PCR AFB –ve: *TCR4* PCR	37 77 77 37 40	0 80 80 0 80	41 1 1 0 1	113 33 33 0 33	None reported
Therese, Jayanthi & Madhavan (2005) India	Level III-2 Some risk of bias	L-J culture	N=9 tissue biopsies n=8	AFB (ZN) IS*6110* PCR AFB + NAAT AFB –ve: IS*6110* PCR	1 1 1 0	0 4 4 4	0 0 0 0	8 4 4 4	None reported
Keys et al. (2012) Australia	Level III-2 Some risk of bias	Culture	N=5 pleural biopsies from children	AFB PCR AFB + NAAT	1 3	0 2	2 0	2 0	1/6 specimens had an inconclusive PCR result

AFB = acid-fast bacilli; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; M-PCR = multiplex PCR; NAAT = nucleic acid amplification test; nPCR = nested PCR; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 89 Diagnostic accuracy of Xpert NAAT compared with AFB microscopy and culture in tissue biopsies

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Al-Atteah et al. (2012) Kingdom of Saudi Arabia	Level III-2 Low risk of bias	MGIT and/or L-J culture	N=16 tissue biopsies	AFB (FL) Xpert	2 8	0 0	6 1	8 7	None reported
Malbruny et al. (2011) France	Level III-2 Low risk of bias	MGIT and Coletsos slants	N=6 vertebral biopsies	AFB (FL) Xpert AFB + NAAT	1 1 1	0 0 0	0 0 0	5 5 5	None reported
Moure, Martin & Alcaide (2012) Spain	Level III-2 Some risk of bias	MGIT and/or L-J culture	N=20 tissue biopsies	AFB –ve: Xpert	5	0	7	8	None reported

AFB = acid-fast bacilli; FL = fluorescent; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF NAAT

Table 90 Diagnostic accuracy of NAAT compared with drug sensitivity testing for the detection of drug-resistant MTB infections

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Carriquiry et al. (2012) Peru	Level III-1 Low risk of bias	DST	N= 39 culture-positive sputum samples	Xpert RIF	6	3	0	30	None reported
Vadwai et al. (2011) India	Level III-2 Low risk of bias	DST with MGIT SIRE	N=125 culture-positive extrapulmonary specimens	Xpert RIF	39	5	1	80	None reported
Kurbatova et al. (2013) Russia	Level III-2 Low risk of bias	DST	N=99 culture-positive Xpert-valid sputum specimens	Xpert (conc) vs L-J or MGIT Xpert (conc) vs L-J DST Xpert (conc) vs MGIT DST	57 45 55	1 2 2	5 5 1	38 30 40	10/109 specimens had invalid Xpert RIF results 2/109 specimens had contaminated MGIT DST 25/109 specimens had contaminated L-J DST

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Bates et al. (2013) Zambia	Level III-2 Low risk of bias	DST with MGIT SIRE	N=52 culture-positive gastric lavage specimens	<u>RIF resistance</u> Xpert RIF <u>Multi- drug resistance</u> Xpert RIF	2 2	1 1	0 0	49 49	6/788 specimens were contaminated and excluded from analysis
Lee et al. (2013) Korea	Level III-2 Low risk of bias	DST on Ogawa medium	N=35 culture-positive Xpert-positive bronchoscopy samples	Xpert RIF	2	0	0	33	None reported
Ioannidis et al. (2011) Greece	Level III-2 Low risk of bias	GenoType MTBDRplus and confirmed by DST	N=32 culture-positive, GeneXpert-valid mixed samples	Xpert RIF	3	1	0	28	None reported
Hillemann et al. (2011) Germany	Level III-2 Low risk of bias	DST	N=29 culture-positive non-respiratory specimens	Xpert RIF	0	1	0	28	3/29 culture-positive specimens by Xpert NAAT had an indeterminate RIF resistance result
Theron et al. (2013) South Africa	Level III-2 Low risk of bias	DST with MGIT SIRE	N=27 culture-positive BAL samples	<u>RIF resistance</u> Xpert RIF <u>MDR</u> Xpert RIF	1 1	3 3	0 0	23 23	None reported
Deggim et al. (2013) Switzerland	Level III-2 Low risk of bias	BACTEC MGIT 960	N=18 culture-positive mixed specimens	Xpert RIF	0	2	0	16	None reported
Sohn et al. (2014) Canada	Level III-2 Some risk of bias	DST	N=501 culture-positive sputum samples	Xpert RIF	1	1	0	499	1/502 specimens had contaminated cultures 44/502 had invalid Xpert results and were repeated
Halse et al. (2010) USA	Level III-2 Some risk of bias	DST	N=143 culture-positive mixed specimens	*rpoB* pyrosequencing	2	1	1	139	None reported

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Inconclusive results
Marlowe et al. (2011) USA	Level III-2 Some risk of bias	DST	N=130 culture-positive respiratory specimens	Xpert RIF	0	3	0	127	None reported
Zeka, Tasbakan & Cavusoglu (2011) Turkey	Level III-2 Some risk of bias	DST	N=89 culture-positive mixed specimens N=58 pulmonary N=31 extrapulmonary	RIF resistance Xpert RIF MDR Xpert RIF	1 1	0 0	0 4	88 84	None reported
Biadglegne et al. (2014) Ethiopia and Germany	Level III-2 Some risk of bias	BacT/Alert 3D DST	N=32 culture-positive FNA samples	Xpert RIF	2	1	0	29	None reported

BAL = bronchoalveolar lavage; DST = drug susceptibility testing; FNA = fine-needle aspirate; L-J = Lowenstein-Jensen; MDR = multidrug-resistant; MGIT = Mycobacterium Growth Indicator Tubes; RIF = rifampicin

Table 91 Diagnostic accuracy of NAAT and AFB microscopy using culture as the reference standard for diagnosis of NTM

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Notes
Abdalla et al. (2009) Brazil	Level II High risk of bias	L-J culture	N=27 FFPE skin biopsy MTB culture-negative specimens	AFB –ve In-house NTM-NAAT	1	13	0	13	Culture not performed on 3/34 specimens 4/34 were MTB C+
Bogner et al. (1997) Germany	Level III-1 Low risk of bias	BACTEC 12B and/or L-J culture	N=540 blood specimens	HIV+ In-house MAC-NAAT	12	2	34	492	Only MAC-positive and MAC-negative results considered Results include 6 MTB and 18 non-MAC NTM C+ specimens

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests	True-positive results	False-positive results	False-negative results	True-negative results	Notes
Mahaisavariya et al. (2005) Thailand	Level III-1 High risk of bias	Culture	N=131 tissue biopsies 5 n=41 6 n=90	AFB In-house NTM-NAAT AFB +ve In-house MTM-NAAT AFB –ve In-house MTM-NAAT	15 16 7 9	26 31 5 26	21 20 8 12	69 64 21 43	11/131 specimens had no culture results and were included as culture-negative Results includes 2 MTB C+ specimens
Kox et al. (1997) The Netherlands	Level III-2 Low risk of bias	Culture	N=238 MTB culture-negative specimens n=53 n=185	AFB (ZN) In-house NTM-NAAT AFB +ve In-house NTM-NAAT AFB –ve In-house NTM-NAAT	32 37 31 6	22 27 17 10	7 2 1 1	177 172 4 168	21/259 specimens were MTB C+
Matsumoto et al. (1998) Japan	Level III-2 Low risk of bias	Ogawa culture	N=139 MTB culture-negative bronchial washings n=133	AFB (ZN) Comm MAC-NAAT AFB –ve Comm MAC-NAAT	4 10 6	2 1 1	8 2 2	125 126 124	2/141 specimens were MTB C+
Choi et al. (2012) Korea	Level III-2 Some risk of bias	MGIT culture	N=467 respiratory specimens that were MTB culture-negative	AFB (FL) Comm NTM-NAAT	6 29	0 0	36 13	425 453	64/531 specimens from patients suspected of MTB infections
Tran et al. (2014) USA	Level III-2 Some risk of bias	BACTEC MGIT 960 culture	N=456 respiratory specimens n=179 n=277	AFB (ZN) In-house MAC-NAAT AFB +ve In-house MAC-NAAT AFB –ve In-house MAC-NAAT	67 57 54 3	112 2 0 2	29 39 13 26	248 358 112 246	8/464 specimens had inconclusive or indeterminate PCR results 25/360 MAC culture-negative specimens grew other NTMs and 101/360 grew MTB but were included as culture-negative in the analysis
Gamboa et al. (1997) Spain	Level III-2 Some risk of bias	BACTEC 13A culture	N=101 specimens N=91 blood specimens N=10 BM specimens	HIV+, AFB –ve Comm MAC-NAAT Comm MAC-NAAT	 57 56	 0 0	 11 10	 33 25	30/121 blood specimens were MTB culture-positive 5/15 BM specimens

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests		True-positive results	False-positive results	False-negative results	True-negative results	Notes
					Comm MAC-NAAT	1	0	1	8	were MTB C+ 6/11 false-negative specimens were non-MAC NTM C+
Ninet et al. (1997) Switzerland	Level III-2 High risk of bias	BACTEC 13A culture	N=195 MTB culture-negative blood specimens	HIV+	Comm MAC-NAAT	14	0	24	157	6/201 were MTB C+ 4/24 false-negative specimens were non-MAC NTM C+
Frevel et al. (1999) Germany	Level III-2 High risk of bias	Culture	N=69 FFPE samples		In-house NTM-NAAT	5	14	0	50	Only NTM results considered MTB culture result unknown

AFB = acid-fast bacilli; BM = bone marrow; C+ = culture positive; Comm = commercial; FFPE = formalin fixed, paraffin embedded; FL = fluorescent; HIV = human immunodeficiency virus; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Table 92 Diagnostic accuracy of NAAT, AFB microscopy and culture using a clinical reference standard for diagnosis of NTM

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests		True-positive results	False-positive results	False-negative results	True-negative results	Notes
Abdalla et al. (2009) Brazil	Level II High risk of bias	CRS	N=27 FFPE skin biopsy MTB culture-negative specimens	AFB –ve	In-house NTM-NAAT Culture	12 1	2 0	0 11	13 15	Culture not performed on 3/34 specimens 4/34 were MTB culture-positive
Phillips et al. (2005) Ghana	Level III-2 Low risk of bias	CRS	Biopsy specimens N=65 N=70 N=65		 AFB (ZN) In-house MU-NAAT Culture	 23 59 27	 0 0 0	 32 1 28	 10 10 10	5/70 specimens did not have microscopy and culture results and were excluded from the analysis
Kox et al. (1997) The Netherlands	Level III-2 Some risk of bias	CRS	N=238 MTB culture-negative specimens		In-house NTM-NAAT Culture	74 38	5 0	2 37	157 162	21/259 specimens were MTB culture-positive

Study Country	Evidence level and risk of bias	Reference standard	Population Samples	Tests		True-positive results	False-positive results	False-negative results	True-negative results	Notes
Gazzola et al. (2008) Italy	Level III-2 Some risk of bias	CRS	N=110 MTB culture-negative specimens N=67 blood specimens N=43 BM specimens	HIV+	MAC-NAAT	10	1	8	91	4/71 blood specimens were MTB culture-positive 3/46 BM specimens were MTB culture-positive
					Culture	13	0	9	92	
					MAC-NAAT	6	0	5	56	
					Culture	10	0	5	56	
					MAC-NAAT + culture	10	0	1	56	
					AFB	1	0	6	36	
					MAC-NAAT	4	1	3	35	
					Culture	3	0	4	36	
					MAC-NAAT + culture	6	1	1	35	

AFB = acid-fast bacilli; BM = bone marrow; Comm = commercial; CRS = clinical reference standard; FFPE = formalin fixed, paraffin embedded; HIV = human immunodeficiency virus; MTB = *Mycobacterium tuberculosis*; MU = *M. ulcerans*; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; PCR = polymerase chain reaction

Appendix D Analysis of diagnostic accuracy data

Table 93 Prevalence of MTB culture-positive patients in included studies

		Overall [range]	> 100 [range]	100–10 [range]	< 10 [range]
All studies					
All NAAT					
Overall	k=68	30% [1–81]	33% [2–81]	29% [1–71]	24% [6–60]
AFB +ve	k=28	80% [27–100]	81% [27–100]	82% [47–98]	72% [54–88]
AFB –ve	k=39	19% [1–72]	18% [1–50]	23% [2–72]	15% [4-48]
HIV+	k=7	30% [16–42]	30% [16–42]	30% [22–34]	-
HIV+, AFB –ve	k=2	15% [12–19]	15% [12–19]	-	-
HIV–	k=6	38% [8–77]	38% [8–77]	-	-
HIV–, AFB +ve	k=4	75% [27–100]	75% [27–100]	-	-
HIV–, AFB –ve	k=5	29% [6–50]	29% [6–50]	-	-
In-house NAAT					
Overall	k=44	32% [1–81]	36% [2–81]	29% [1–71]	24% [12–60]
AFB +ve	k=16	80% [47–100]	86% [65–100]	74% [47–98]	69% [54–84]
AFB –ve	k=23	17% [1-44]	20% [1–44]	13% [2–31]	5% [4–5]
Xpert					
Overall	k=24	27% [5–77]	27% [5–77]	29% [8–50]	24% [6–60]
AFB +ve	k=12	78% [27–100]	67% [27–100]	92% [64–98]	74% [64–88]
AFB –ve	k=16	23% [1–72]	14% [1–50]	37% [10-72]	18% [4–48]
Sputum specimens					
All NAAT					
Overall	k=30	37% [5–81]	42% [7–81]	38% [5–71]	15% [6–28]
AFB +ve	k=14	76% [27–100]	78% [27–100]	80% [47–100]	59% [54–64]
AFB –ve	k=16	25% [4–100]	34% [9–100]	24% [4–56]	5% [4–6]
HIV+	k=3	35% [34–38]	38% (k=1)	34% [34–34]	-
HIV+, AFB +ve	k=1	12%	12%	-	-
HIV–	k=2	42% [8–77]	42% [8–77]	-	-

		Overall [range]	> 100 [range]	100–10 [range]	< 10 [range]
HIV–, AFB +ve	k=1	27%	27%	-	-
HIV–, AFB –ve	k=1	50%	50%	-	-
In-house NAAT					
Overall	k=18	41% [5–81]	58% [41–81]	34% [1–71]	13% [12–15]
AFB +ve	k=9	76% [47–98]	85% [65–95]	72% [47–98]	54% (k=1)
AFB –ve	k=10	26% [4–100]	40% [20–100]	14% [4–24]	5% (k=1)
Xpert					
Overall	k=12	32% [6–77]	26% [7–77]	49% [34–68]	17% [6–28]
AFB +ve	k=5	76% [27–100]	64% [27–100]	96% [91–100]	64% (k=1)
AFB –ve	k=7	23% [4–56]	23% [9–50]	42% [28–56]	5% [4–6]
Non-sputum specimens					
All NAAT					
Overall	k=35	27% [1–67]	29% [2–67]	24% [1–48]	23% [10–60]
AFB +ve	k=11	80% [44–100]	86% [62–100]	68% [44–92]	79% [74–85]
AFB –ve	k=19	18% [0–72]	14% [0–44]	32% [2–72]	9% [7–10]
HIV+	k=4	26% [16–42]	27% [16–42]	30% [22–34]	-
HIV+, AFB –ve	k=1	19%	19%]	-	-
HIV–	k=3	34% [14–46]	34% [14–46]	-	-
HIV–, AFB +ve	k=2	94% [88–100]	94% [88–100]	-	-
HIV–, AFB –ve	k=3	24% [6–38]	24% [6–38]	-	-
In-house NAAT					
Overall	k=25	27% [1–67]	29% [2–67]	21% [1–40]	27% [10–60]
AFB +ve	k=8	79% [44–100]	86% [62–100]	44% (k=1)	74% (k=1)
AFB –ve	k=13	15% [0–44]	15% [0–44]	19% [2–32]	7% (k=1)
Xpert					
Overall	k=10	26% [6–60]	28% [6–60]	28% [13–48]	16% [16–16]
AFB +ve	k=4	84% [67–92]	92% (k=1)	76% [67–85]	92% (k=1)
AFB –ve	k=6	24% [1–72]	6% [1–10]	41% [8–72]	10% (k=1)

AFB = acid-fast bacilli; HIV = human immunodeficiency virus; MTB = *Mycobacterium tuberculosis*; NAAT = nucleic acid amplification test; Xpert = GeneXpert MTB/RIF NAAT

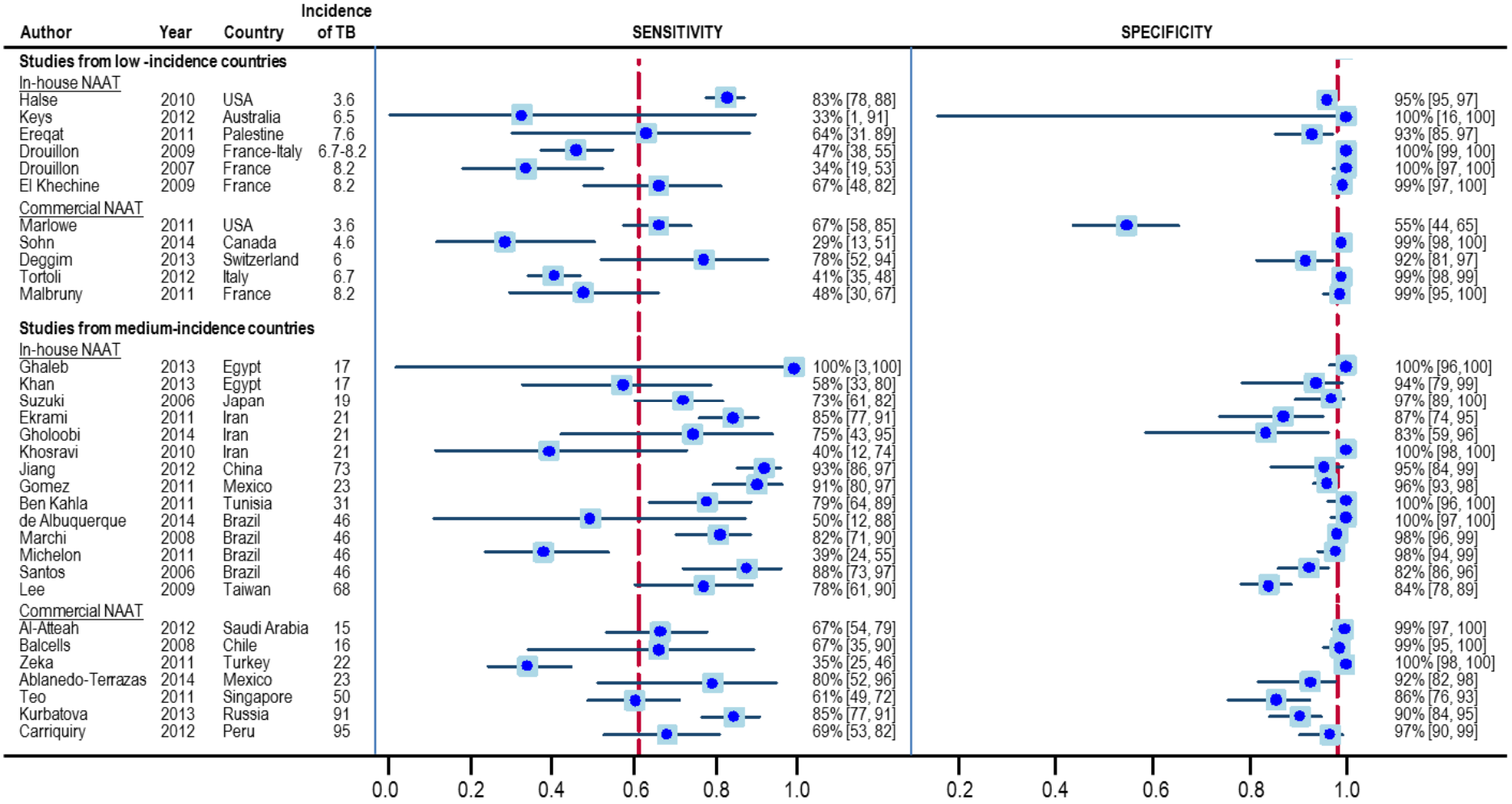

Figure 38 Forest plot of the sensitivity and specificity of AFB microscopy compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with low and medium incidence of TB

Incidence of TB based on WHO estimates from 2012: low incidence = ≤ 10 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

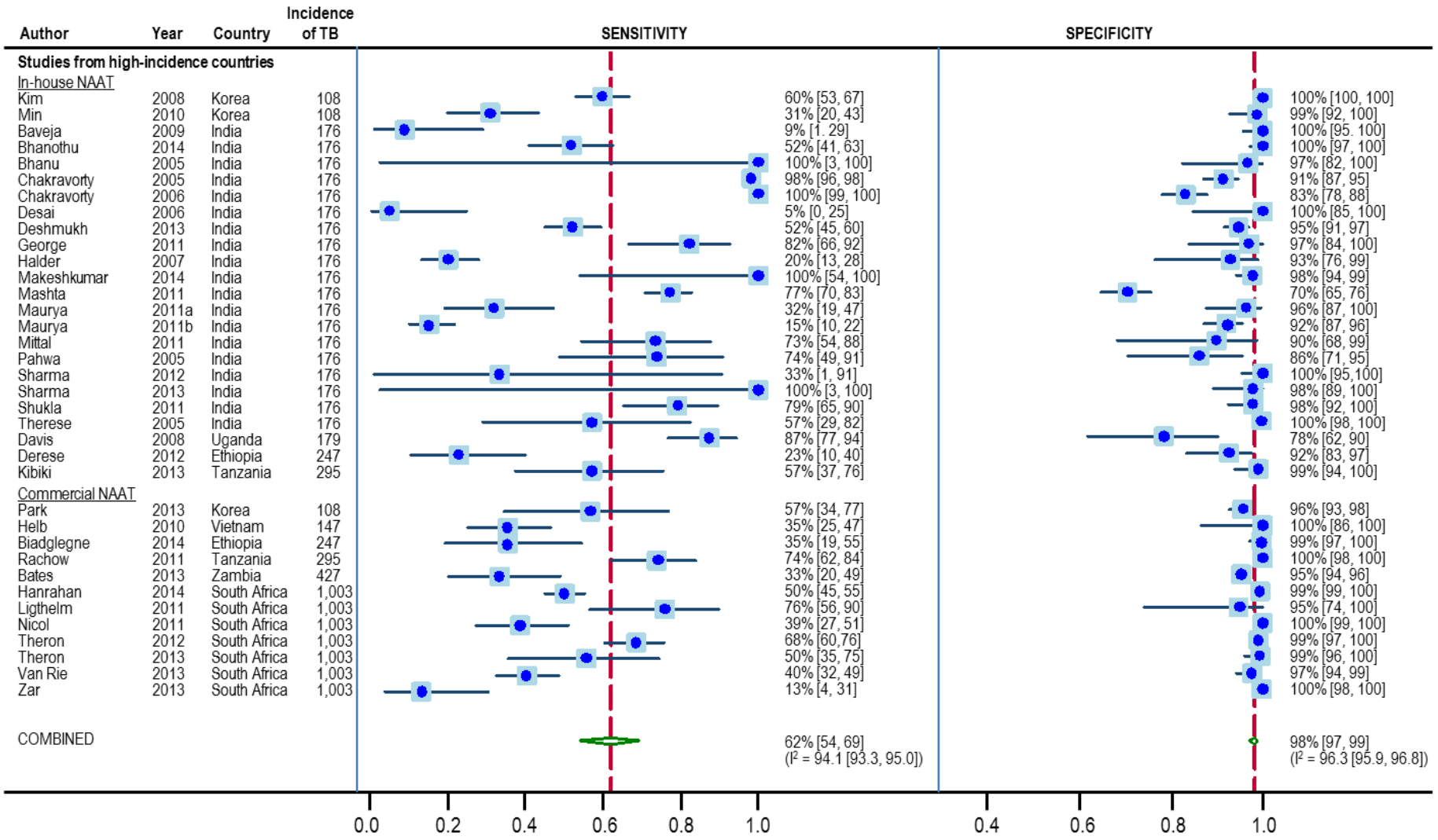

Figure 39 Forest plot of the sensitivity and specificity of AFB microscopy compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with high incidence of TB

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people. The combined values are for all studies in both Figures 38 and 39

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 94 Pooled sensitivity and specificity of AFB and NAAT in non-sputum specimens compared with extrapulmonary specimens

	Non-sputum Sensitivity [95%CI]		Non-sputum Specificity [95%CI]		Extrapulmonary Sensitivity [95%CI]		Extrapulmonary Specificity [95%CI]	
AFB								
All NAAT	46% [37, 55]	k=35	98% [97, 99]	k=35	44% [33, 55]	k=29	98% [96, 99]	k=29
In-house NAAT	46% [34, 59]	k=25	98% [96, 99]	k=25	43% [29, 58]	k=21	98% [96, 99]	k=21
Commercial NAAT	46% [35, 57]	k=10	99% [98, 99]	k=10	48% [34, 63]	k=8	91% [81, 96]	k=8
AFB (ZN)	46% [34, 59]	k=26	98% [96, 99]	k=26	46% [31, 61]	k=21	98% [95, 99]	k=21
AFB (FL)	46% [35, 57]	k=10	96% [90, 99]	k=10	42% [32, 53]	k=7	99% [97, 99]	k=7
NAAT								
All NAAT	91% [83, 94]	k=35	92% [84, 96]	k=35	91% [83, 96]	k=29	89% [79, 95]	k=29
In-house NAAT	91% [79, 96]	k=25	88% [75, 95]	k=25	91% [78, 97]	k=21	86% [70, 95]	k=21
Commercial NAAT	90% [83, 94]	k=10	96% [90, 99]	k=10	90% [83, 94]	k=8	92% [85, 96]	k=8
AFB + NAAT								
All NAAT	94% [91, 96]	k=18	83% [70, 91]	k=18	94% [91, 96]	k=17	81% [68, 90]	k=17
In-house NAAT	100% [87, 100]	k=11	56% [11, 93]	k=11	93% [87, 96]	k=10	70% [49, 85]	k=10
Commercial NAAT	94% [90, 96]	k=7	92% [82, 97]	k=7	94% [91, 97]	k=7	91% [81, 96]	k=7

AFB = acid-fast bacilli; CI = confidence interval; FL = fluorescent staining; NAAT = nucleic acid amplification test; TB = tuberculosis; ZN = Ziehl-Neelsen staining

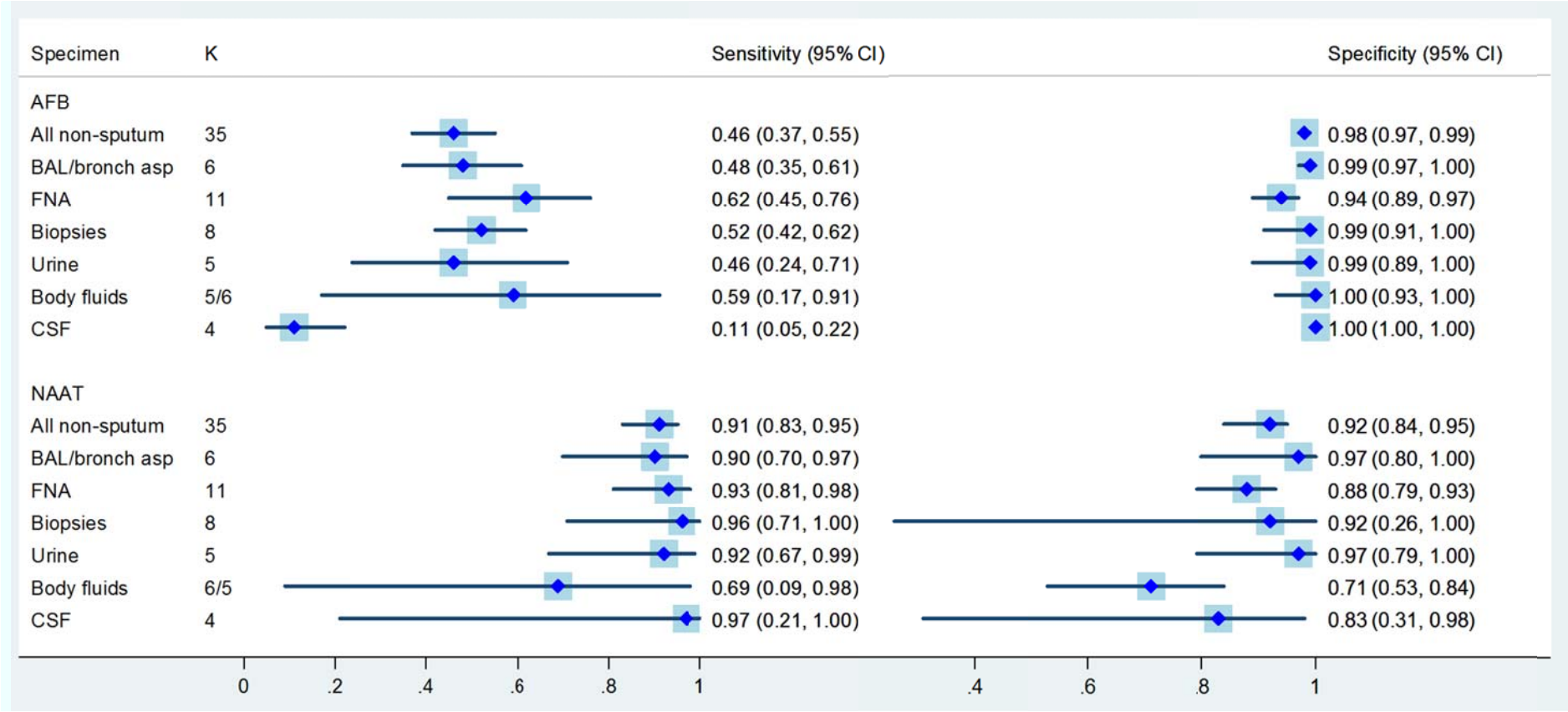

Figure 40 Forest plot showing the pooled sensitivity and specificity values for AFB, NAAT and AFB plus NAAT compared with culture, according to specimen type
AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid; FNA = fine-needle aspirate; NAAT = nucleic acid amplification test

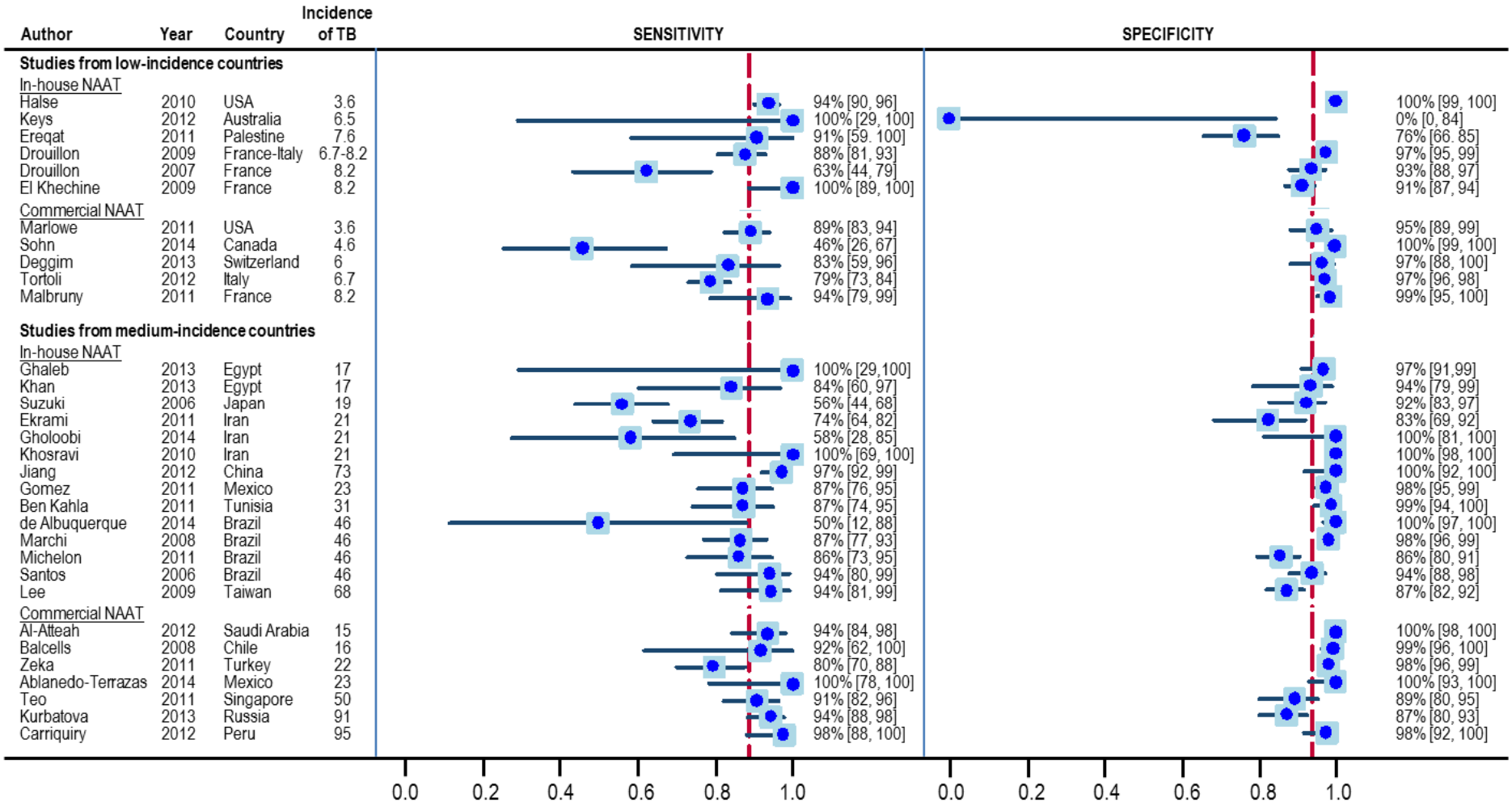

Figure 41 Forest plot of the sensitivity and specificity of NAAT compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with low and medium incidence of TB

Incidence of TB based on WHO estimates from 2012: low incidence = ≤ 10 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people

NAAT = nucleic acid amplification test; TB = tuberculosis

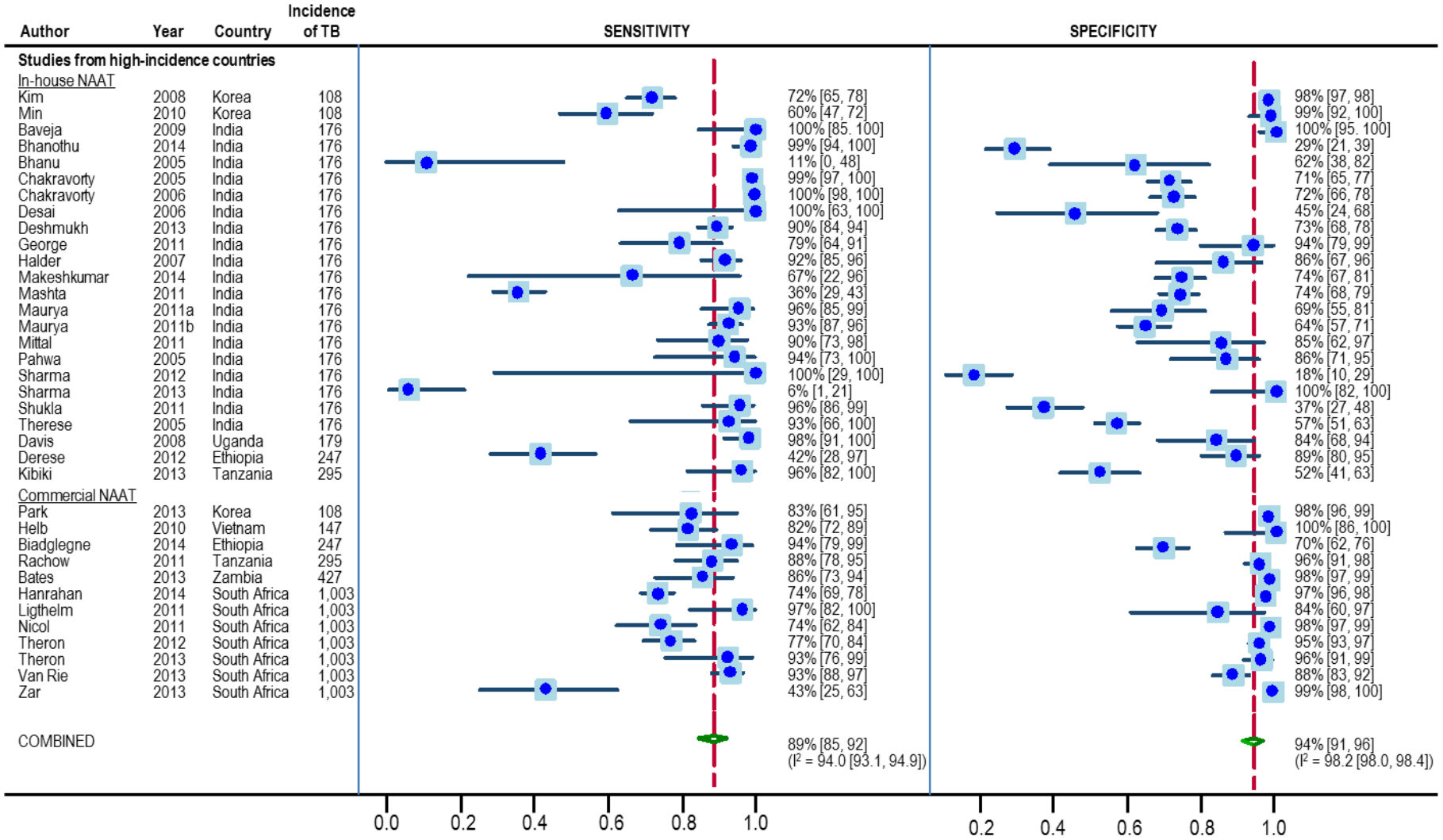

Figure 42 Forest plot of the sensitivity and specificity of NAAT compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with high incidence of TB

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people. The combined values are for all studies in both Figures 41 and 42.

NAAT = nucleic acid amplification test; TB = tuberculosis

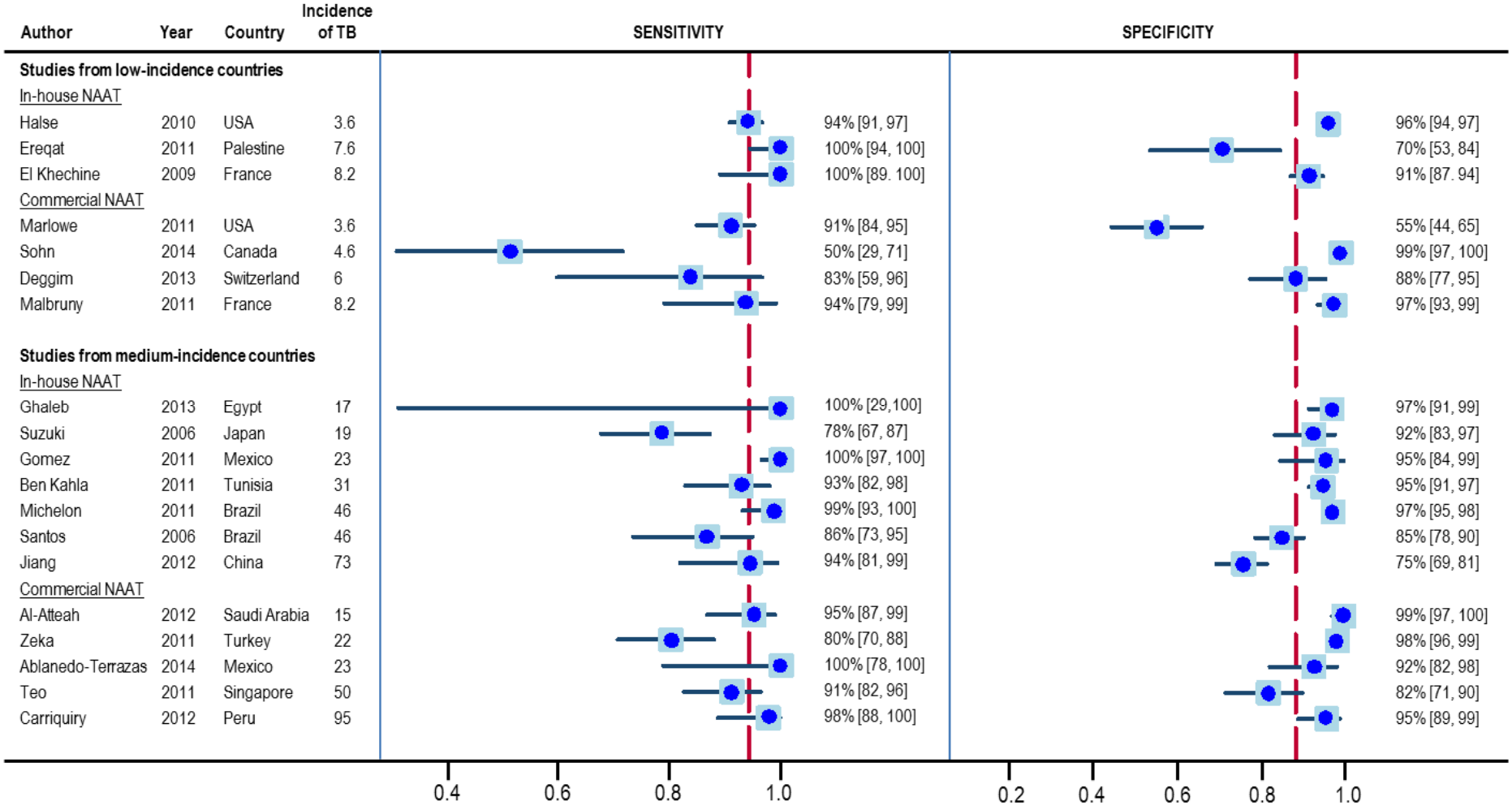

Figure 43 Forest plot of the sensitivity and specificity of AFB plus NAAT compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with low and medium incidence of TB

Incidence of TB based on WHO estimates from 2012: low incidence = ≤ 10 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

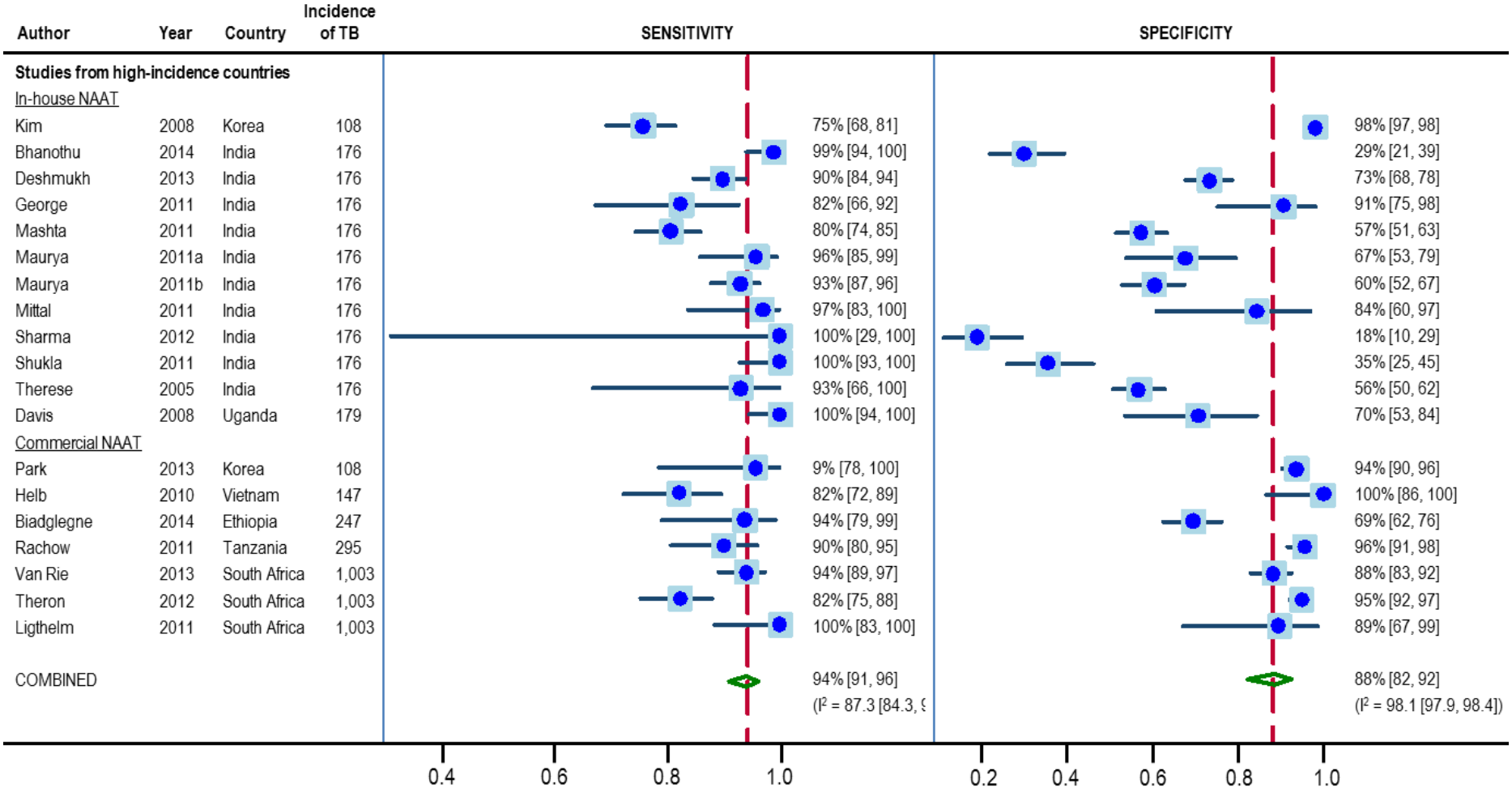

Figure 44 Forest plot of the sensitivity and specificity of AFB plus NAAT compared with culture, grouped according to use of in-house or commercial NAAT, for studies conducted in countries with high incidence of TB

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people. The combined values are for all studies in both Figures 43 and 44.

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

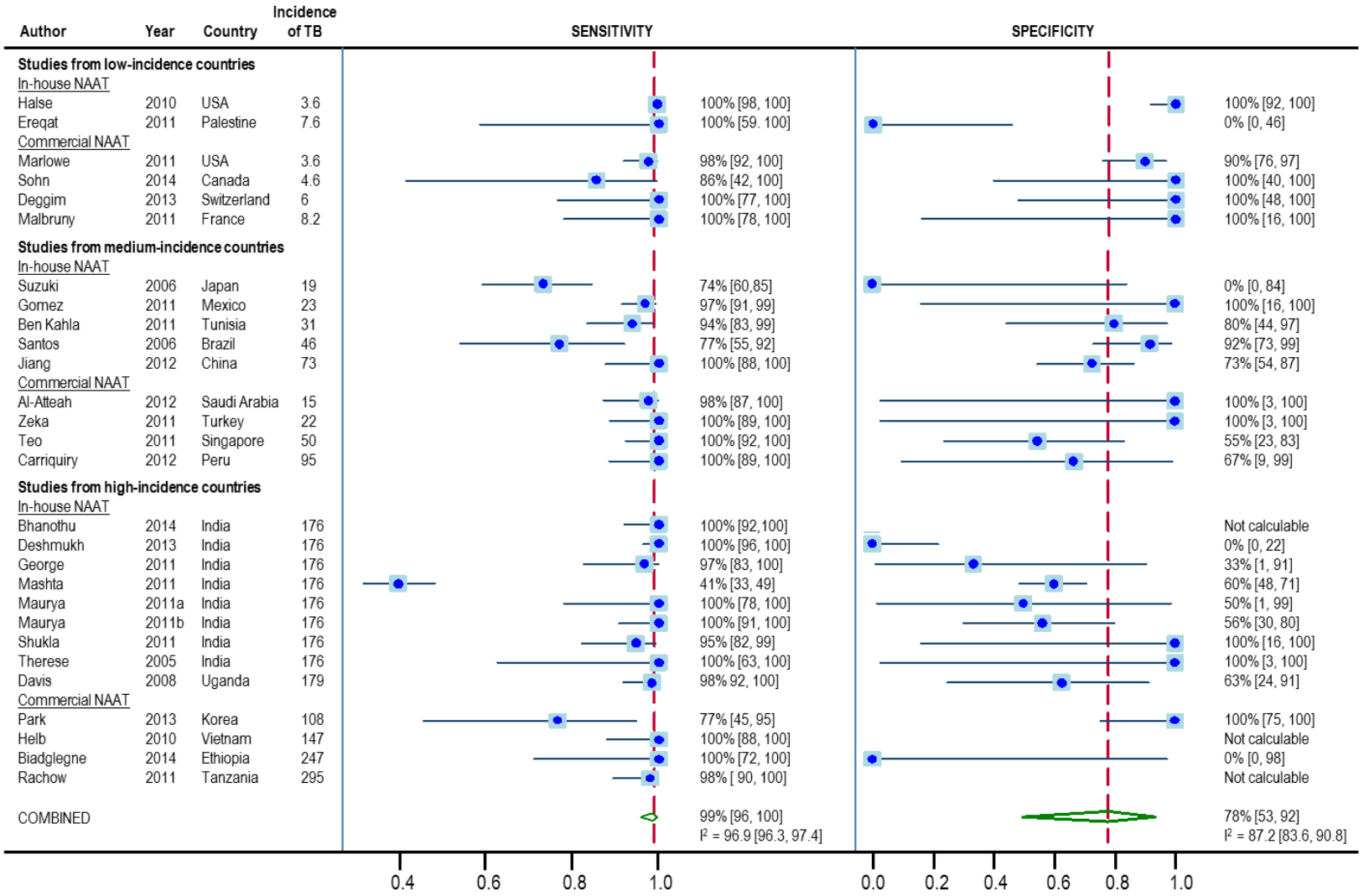

Figure 45 Forest plot of the sensitivity and specificity of NAAT compared with culture in AFB-positive specimens, grouped according to type of NAAT and incidence of TB

Incidence of TB based on WHO estimates from 2012: low incidence = ≤ 10 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; high incidence = > 100 cases per 100,000 people

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

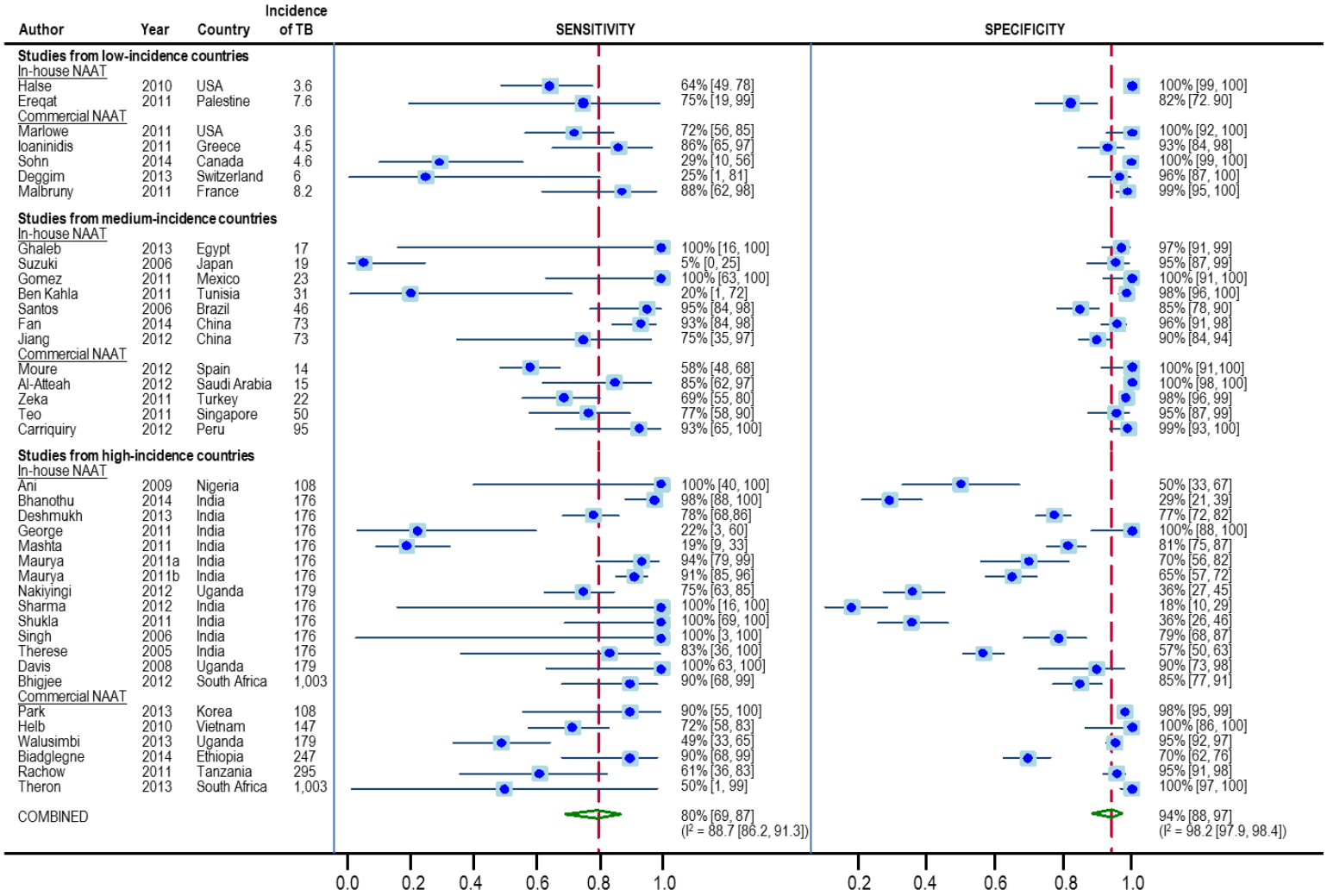

Figure 46 Forest plot of the sensitivity and specificity of NAAT compared with culture in AFB-negative specimens, grouped according to use of in-house or commercial NAAT and incidence of TB

Incidence of TB based on WHO estimates from 2012: low incidence = ≤ 10 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; high incidence = > 100 cases per 100,000 people

AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

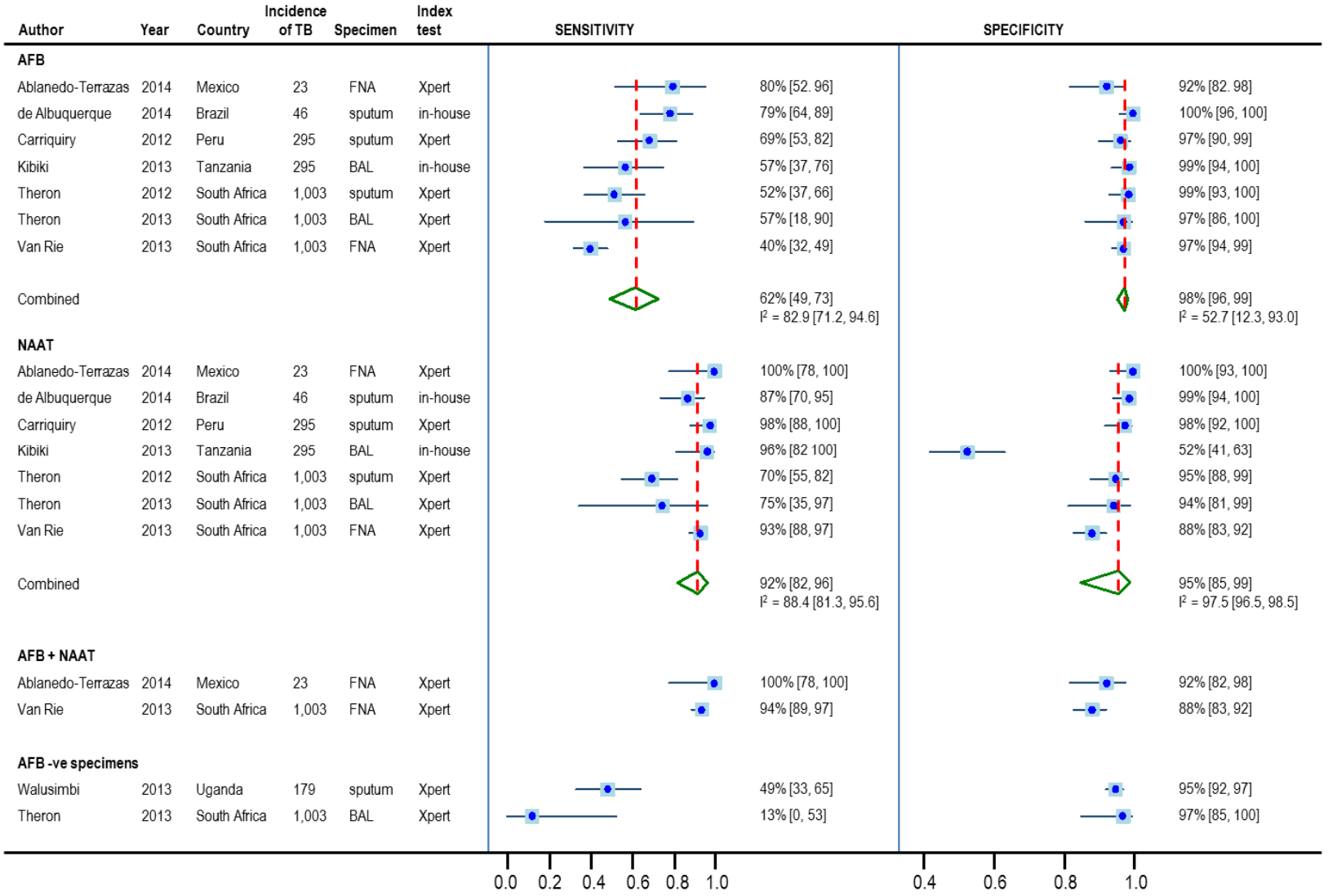

Figure 47 Forest plot of the sensitivity and specificity of AFB and/or NAAT compared with culture in HIV-positive patients suspected of having TB

Incidence of TB based on WHO estimates from 2012

AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; FNA = fine-needle aspirate; NAAT = nucleic acid amplification test; TB = tuberculosis

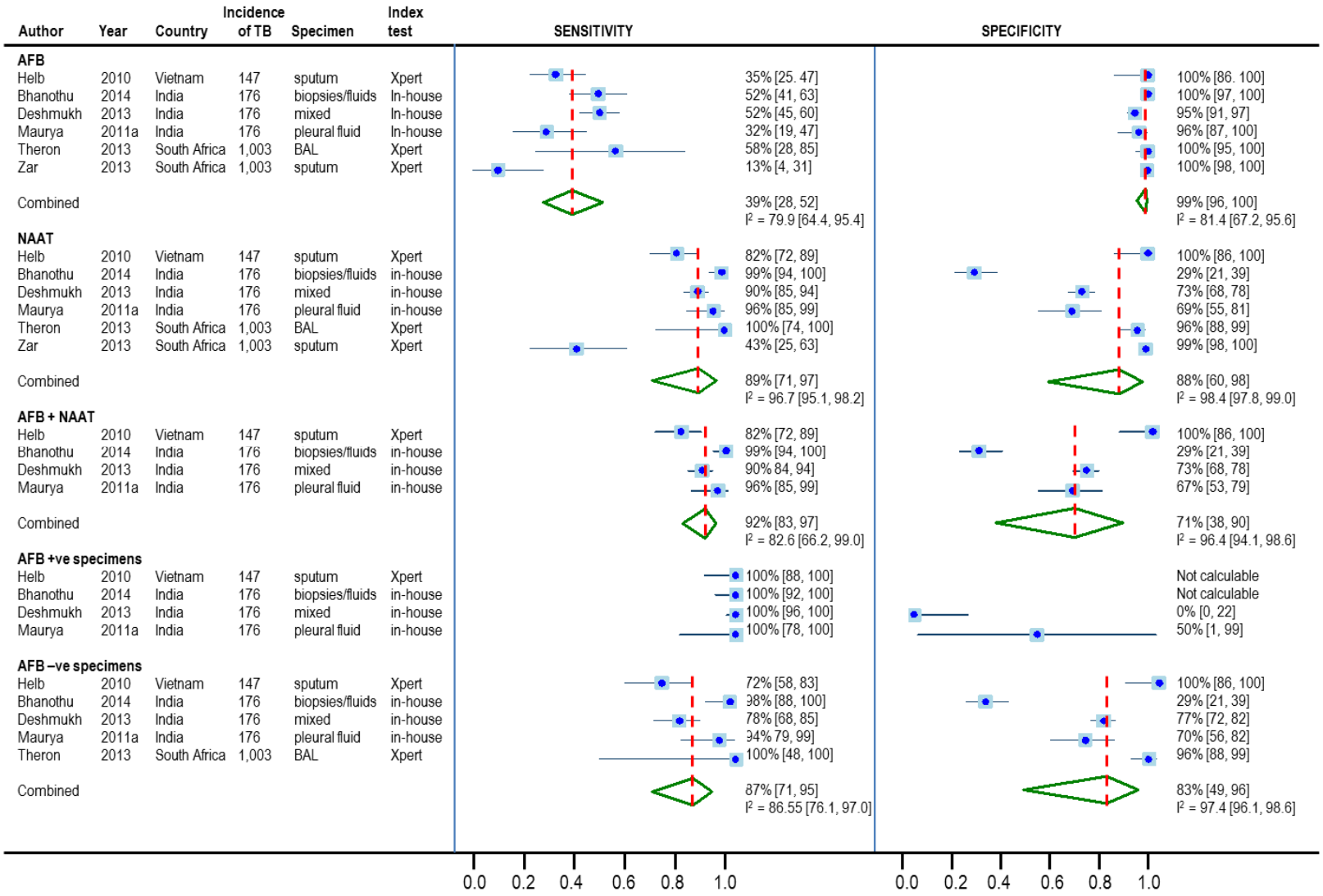

Figure 48 Forest plot of the sensitivity and specificity of AFB and/or NAAT compared with culture in HIV-negative patients suspected of having TB

Incidence of TB based on WHO estimates from 2012

AFB = acid-fast bacilli; BAL = bronchoalveolar lavage; HIV = human immunodeficiency virus; NAAT = nucleic acid amplification test; TB = tuberculosis

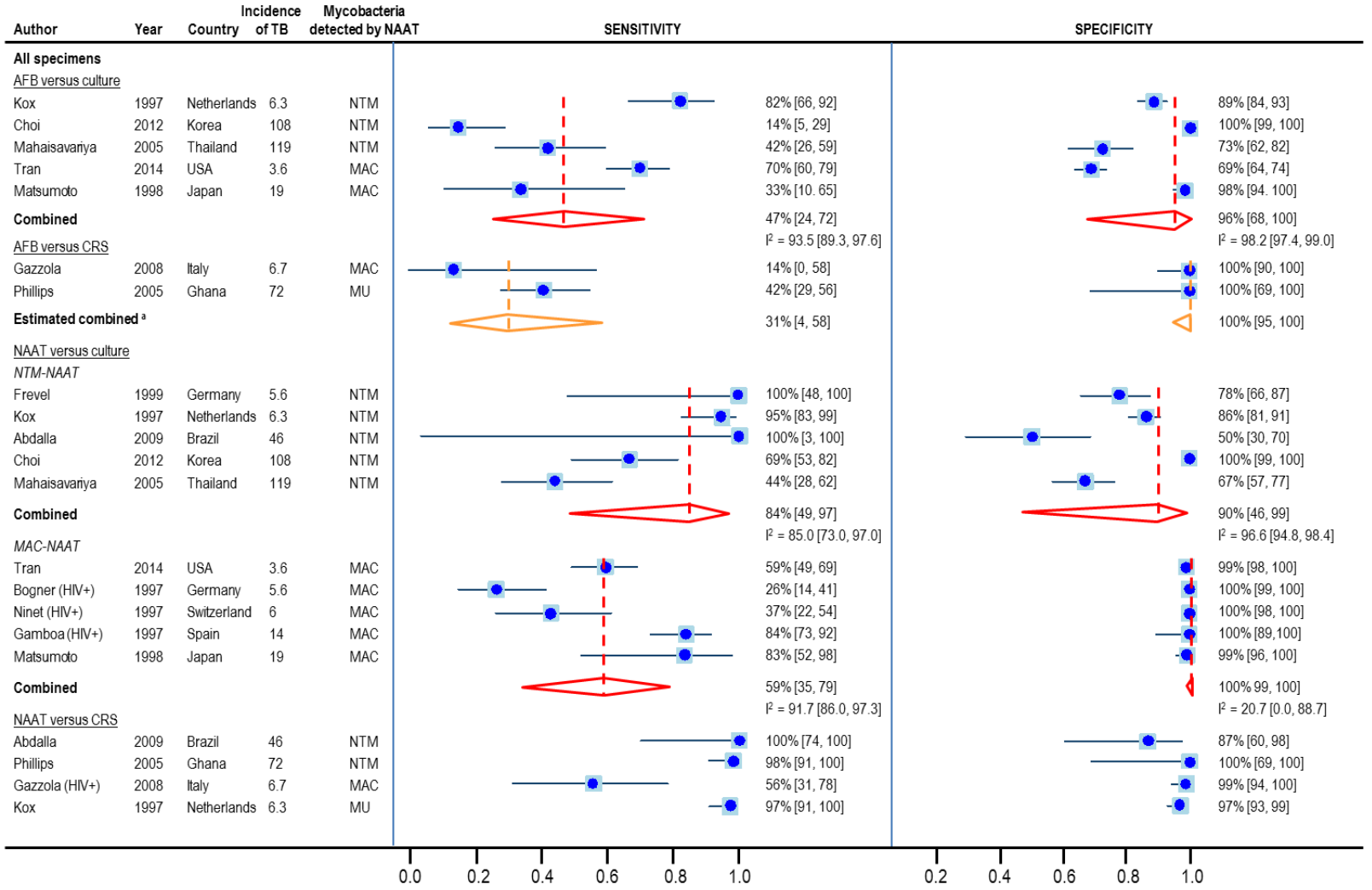

Figure 49 Forest plot of the sensitivity and specificity of AFB and NAAT compared with culture or a clinical reference standard in diagnosing NTM infections

[a] Estimated pooled values were obtained using the metan command in Stata 12.1

Incidence of TB based on WHO estimates from 2012

AFB = acid-fast bacilli; CRS = clinical reference standard; HIV = human immunodeficiency virus; MAC = *Mycobacterium avium* complex; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria

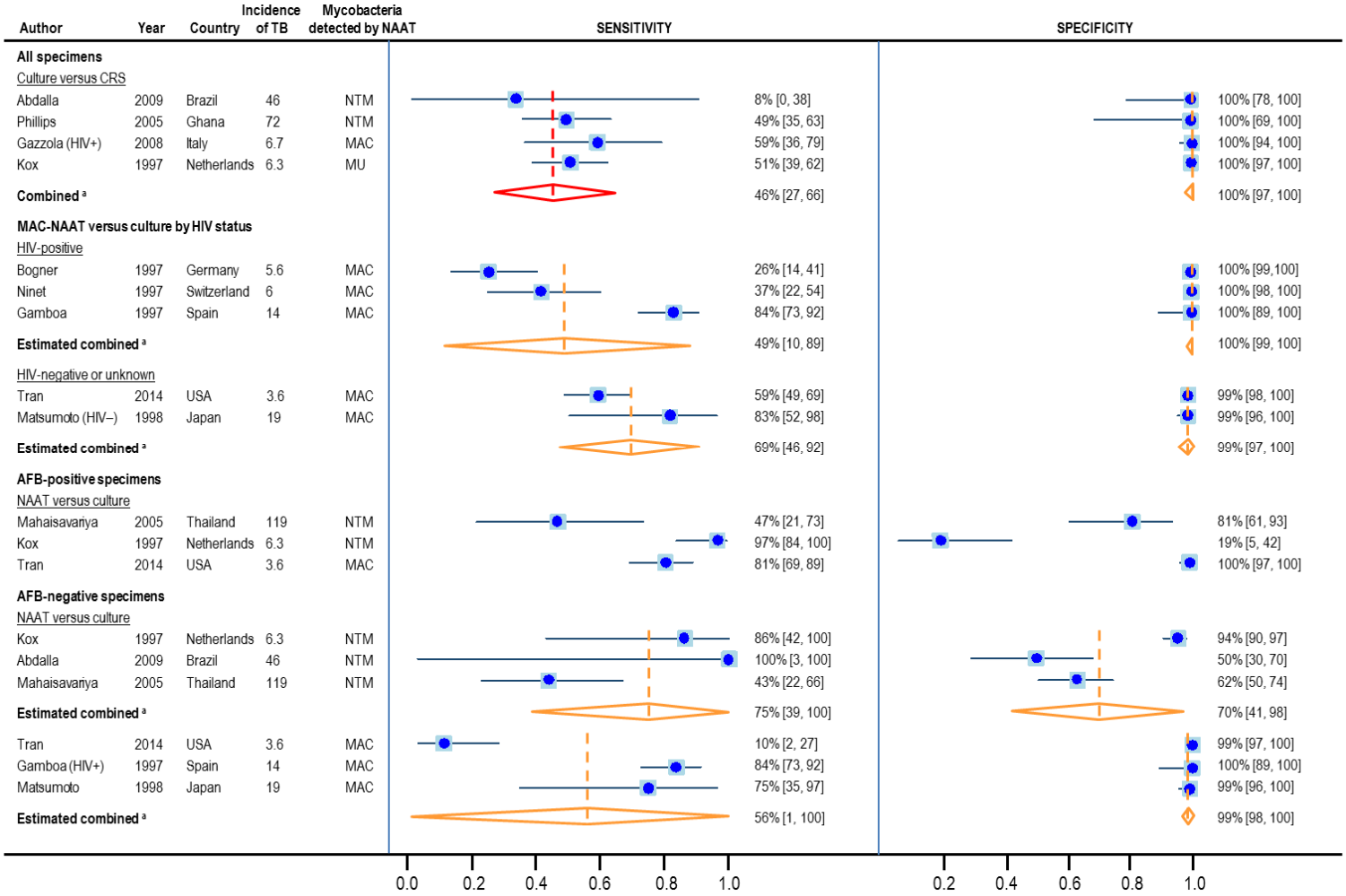

Figure 50 Forest plot of the sensitivity and specificity of culture compared with a clinical reference standard and subgroup analysis of NAAT compared with culture, based on HIV and AFB status

[a] Estimated pooled values were obtained using the metan command in STATA 12.1

Incidence of TB based on WHO estimates from 2012

AFB = acid-fast bacilli; CRS = clinical reference standard; HIV = human immunodeficiency virus; MAC = *Mycobacterium avium* complex; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria.

Appendix E Meta-analysis of studies assessing the diagnostic accuracy of AFB compared with culture

Of the 68 studies that compared the diagnostic accuracy of AFB microscopy to culture in patients suspected of having TB, 39 performed AFB microscopy using ZN staining, 23 used fluorescent stains such as auramine, 2 used alternative stains and 3 did not report the method used. Interestingly, 18/24 (75%) studies comparing AFB microscopy and the Xpert assay used fluorescent staining, whereas 34/44 (77%) of studies using in-house NAAT methods used ZN staining. Forest plots showing the sensitivity and specificity for these studies are shown in Figure 38 and Figure 39 (Appendix D). The sensitivity varied greatly between studies, ranging from 5% to 100% with a pooled sensitivity of 62% (95%CI 54, 69). There was less variability in the specificity, which was above 80% in all but 3 studies, with a pooled value of 98% (95%CI 97, 99). The proportion of culture-positive specimens that were AFB-positive is higher in these studies than that reported in the *Tuberculosis notifications in Australia, 2010 Annual Report*[30], which reported that, of all MTB cases confirmed by culture, only 47% were AFB-positive.

Subgroup analysis was undertaken to determine the effects of AFB methodology, specimen type, incidence of TB in the country in which the study was conducted, and use of in-house or commercial NAAT index test on the accuracy of AFB microscopy (Figure 51). There was a significant difference in sensitivity between studies investigating diagnostic accuracy in patients who provided sputum samples (71%; 95%CI 59, 81) compared with those that provided non-sputum samples (46%; 95%CI 37, 55), as the 95%CIs did not overlap. Non-sputum specimens included patients suspected of having either pulmonary TB (e.g. bronchial aspirates) or extrapulmonary TB (e.g. synovial fluid or tissue biopsy). Analysis of extrapulmonary specimens alone showed that the sensitivity and specificity of AFB compared with culture did not differ markedly from those for non-sputum samples (Table 94 in Appendix D). For some specific specimen types there were sufficient studies for separate analysis (Figure 40 in Appendix D). The pooled sensitivity for AFB microscopy compared with culture varied from 46% in urine to 62% in FNAs of lymph nodes. However,

[30] Available from URL: http://www.health.gov.au/internet/main/publishing.nsf/Content/cda-cdi3801-pdf-cnt.htm/$FILE/cdi3801i.pdf (accessed 3 November 2014)

for CSF the pooled sensitivity was only 11%. Thus, AFB microscopy is not a useful tool for diagnosis of TB in CSF specimens. The pooled specificity was at least 94% in all specimen types.

There was an overall 11% difference in sensitivity of AFB microscopy compared with culture, favouring studies that used an in-house NAAT over those that used the commercial Xpert NAAT, which was not statistically significant. However, this difference was entirely due to the type of specimen tested. In studies that used sputum samples, AFB microscopy was 24% more sensitive compared with culture when an in-house NAAT was used as the index test instead of a commercial NAAT. Conversely, there was no difference in sensitivity in studies that used non-sputum samples (Figure 51).

The reason for this is unclear, although there is likely to be some publication bias, as indicated by the significant asymmetry when comparing the effective sample size between studies (Figure 52). This asymmetry was no longer significant (p>0.05) when the studies were separated according to AFB methodology, NAAT methodology or specimen type (data not shown). Other variables that may influence publication bias include funding, conflict of interest, prejudice against an observed association and sponsorship, but the effects of these parameters were not tested.

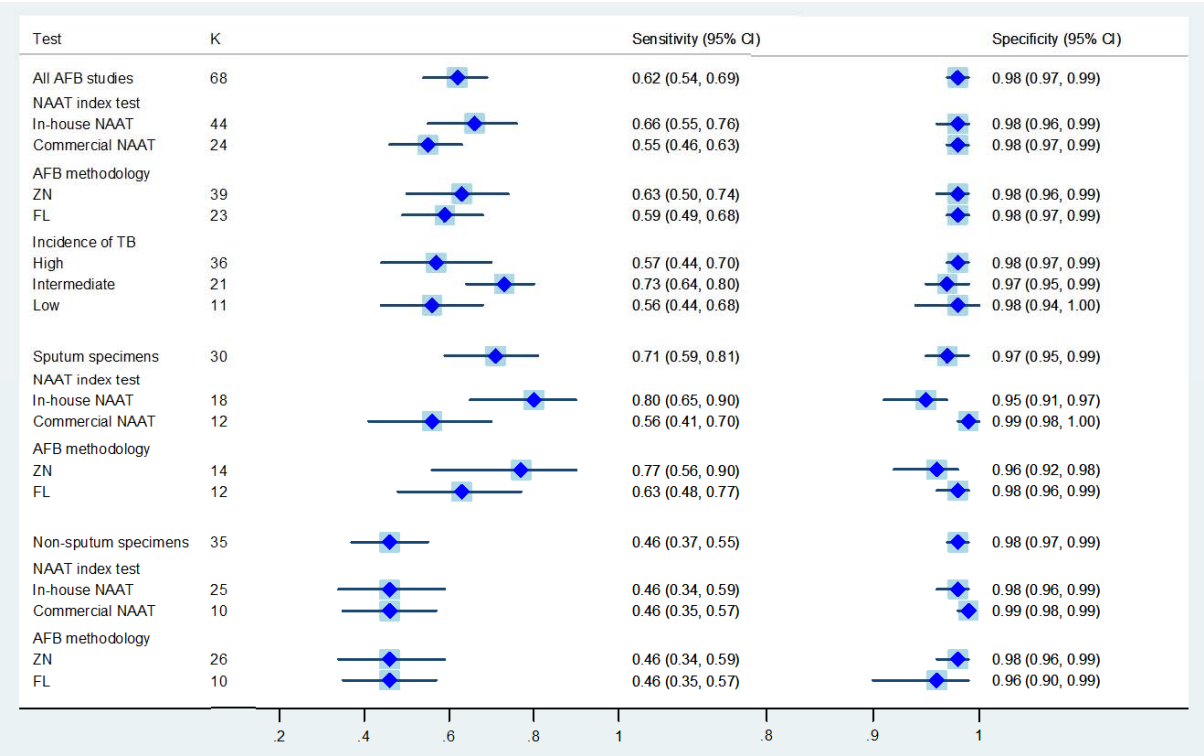

Figure 51 Forest plot showing the pooled sensitivity and specificity values for AFB microscopy compared with culture for studies grouped according to NAAT comparator, AFB methodology and incidence of TB in the country in which the study was conducted

Incidence of TB based on WHO estimates from 2012: high incidence = > 100 cases per 100,000 people; medium incidence = 10–100 cases per 100,000 people; low incidence = ≤ 10 cases per 100,000 people
FL = fluorescent staining; K = the number of studies; NAAT = nucleic acid amplification testing; TB = tuberculosis; ZN = Ziehl-Neelsen staining

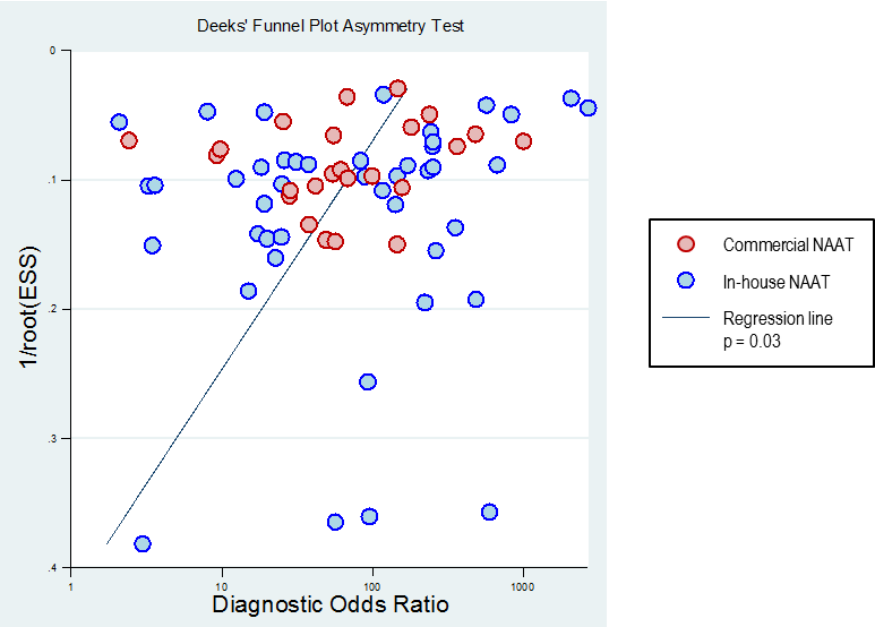

Figure 52 Deek's Funnel plot asymmetry test to assess publication bias for the diagnostic accuracy of AFB microscopy compared with culture

Publication bias is assessed visually by using the inverse of the square root of the effective sample size versus the diagnostic log odds ratio, which should have a symmetrical funnel shape when publication bias is absent (Light & Pillemer 1984). A regression slope coefficient, weighting by ESS, with $p<0.05$ indicates significant asymmetry (Deeks, Macaskill & Irwig 2005).

There was little to no difference in sensitivity and specificity between studies conducted in high-TB-incidence countries compared with low-incidence countries. The anomaly seen for medium-incidence countries was likely due to chance, given the variability between studies, as seen in Figure 38 and Figure 39 (Appendix D).

LR scattergrams plot LR+ against LR– where the likelihood of correctly identifying patients with MTB infections (as diagnosed by culture) increases along the x-axis and the likelihood of correctly eliminating the presence of MTB decreases along the y-axis. The summary LR+ and LR– values for studies investigating the ability of AFB microscopy to correctly diagnose patients with or without TB, compared with culture, were within the upper right quadrant of the graph (Figure 53). This quadrant represents LR+ and LR– values that suggest that AFB microscopy is likely to correctly confirm the presence of MTB, but a negative test result does not eliminate the likelihood of a positive culture result in patients suspected of having TB. The observed difference in sensitivity of AFB microscopy compared with culture in sputum and non-sputum specimens did not affect the clinical utility of the AFB test. The LRs for both sputum (LR+ 27.0 [95% CI 15.9, 45.6]; LR– 0.29 [95%CI 0.20, 0.43] and non-sputum (LR+ 23.3 [95%CI 13.7, 39.7]; LR– 0.55 [95%CI 0.47, 0.65] specimens were also in the same upper right

quadrant. Thus, AFB microscopy is useful for those patients with a positive AFB test result as it identifies those patients as having TB and requiring immediate treatment. However, the clinician gains no further knowledge if a patient has a negative AFB test result, as this patient may still have TB.

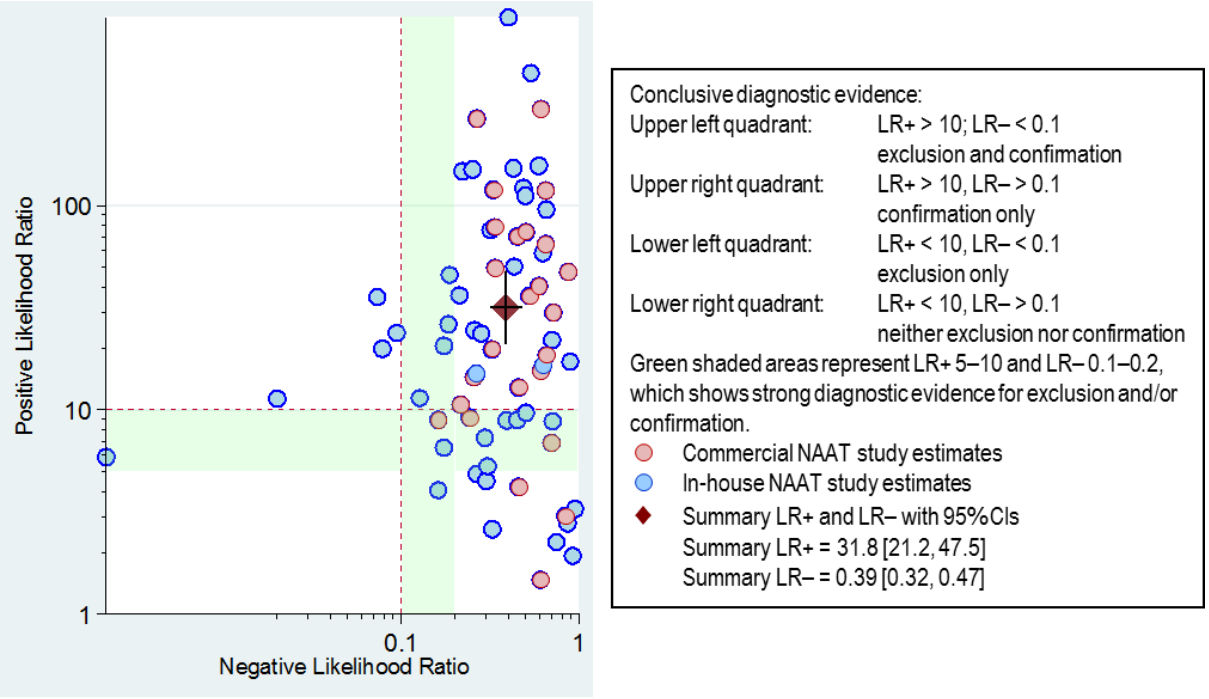

Figure 53 LR scattergram for diagnosis of MTB infection by AFB microscopy compared with culture in studies using in-house NAAT or the Xpert NAAT

LR = likelihood ratio; NAAT = nucleic acid amplification testing

The SROC curve, which depicts the relative trade-off between true-positive and false-positive results, indicated that AFB microscopy performs well in predicting culture positivity, with an AUC of 0.94 (95%CI 0.92, 0.96). There was no threshold effect based on the AFB staining methodology, suggesting that it does not impact on the sensitivity or specificity of AFB microscopy when compared with culture (Figure 54). This lack of threshold effect suggests that the observed differences in sensitivity between studies using in-house NAATs (which favoured ZN staining) and commercial NAATs (which favoured fluorescent staining) were due to other differences that have not been identified. However, there was a threshold effect based on specimen type, with sensitivity being higher when sputum specimens were tested (Figure 54).

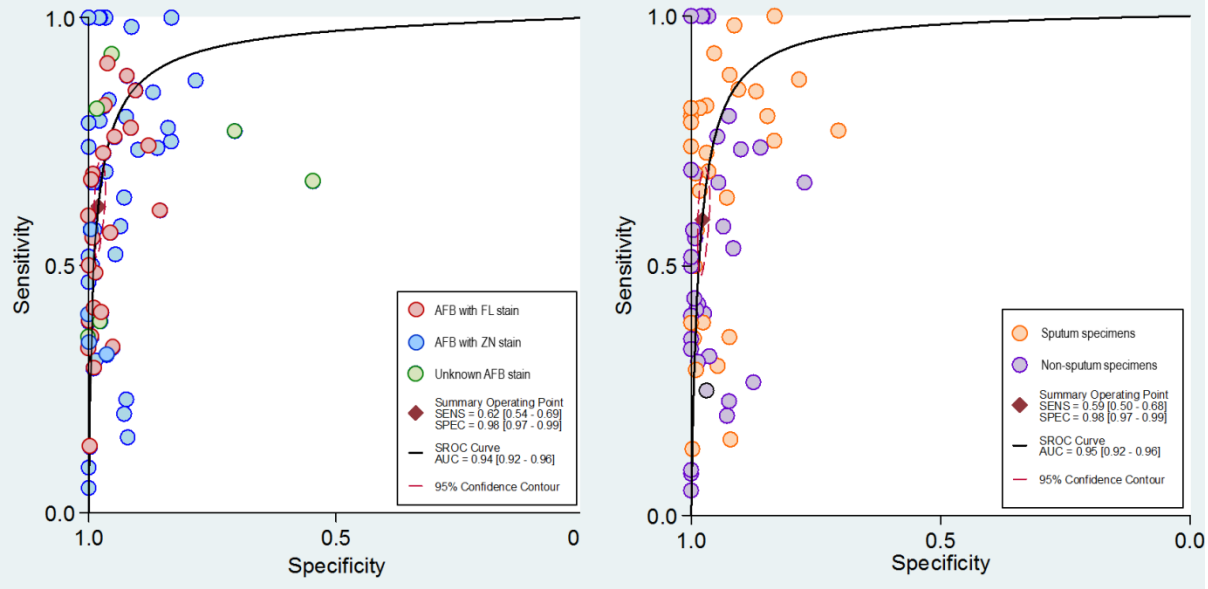

Figure 54 SROC curve for all studies investigating the sensitivity and specificity of AFB microscopy versus culture in the diagnosis of TB

AFB = acid-fast bacilli; AUC = area under curve; FL = fluorescent staining; SROC = summary receiver–operator characteristic; NAAT = nucleic acid amplification testing; ZN = Ziehl-Neelsen staining

Appendix F Study profiles of studies included in the assessment

Table 95 Study profiles of included studies providing direct evidence on the effectiveness of NAAT on patients suspected of having TB

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Theron et al. (2014) University of Cape Town, South Africa Conducted at: Five primary healthcare facilities in areas with a high HIV prevalence in South Africa, Zimbabwe and Tanzania	Randomised controlled trial (multicentre) Level: II Quality: 23/26 Low risk of bias	N=1,502 Median age: 37 years (IQR 30–46), 643 (43%) females, 895 (60%) HIV infected 758 assigned to AFB microscopy 744 assigned to Xpert MTB/RIF	Inclusion: > 17 years of age, one or more symptoms of pulmonary TB (according to WHO criteria), able to provide sputum specimens, no anti-TB treatment in past 60 days Exclusion: Not reported	Xpert MTB/RIF on sputum specimen by nurse who received a 1-day training session	AFB microscopy on sputum specimen Positive if any smear revealed AFB over 100 fields (1000x for light microscopy and 400x for fluorescence microscopy)	TB-related morbidity after 2 and 6 months (using TBscore and Karnofsky performance score) Mortality at 6-month follow-up Failure rates
Yoon et al. (2012) Division of Pulmonary and Critical care Medicine, San Francisco General Hospital, University of San Francisco, San Francisco, California, USA Conducted at: Mulago Hospital, Kampala, Uganda	Historical cohort study Level: III-3 Quality: 18.5/26 Some risk of bias	N=477/525 included Median age: 33 years (IQR 27–40), 229 (48%) female, 362 (76%) HIV infected	Inclusion: Consecutive adults > 17 years of age admitted to hospital with cough > 2 weeks but < 6 months duration and provided consent Exclusion: Receiving TB treatment at the time of enrolment, no available culture results, no NAAT on implementation phase, death within 3 days of hospital admission	GeneXpert MTB/RIF, sputum AFB microscopy and mycobacterial culture	Same tests, but in comparator group Xpert results were not reported to clinicians or used for patient management	2-month mortality

IQR = interquartile range; NAAT = nucleic acid amplification test; TB = tuberculosis; WHO = World Health Organization

Table 96 Study profiles of included studies on diagnostic accuracy

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Ablanedo-Terrazas et al. (2014) Mexico	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=68 lymph node FNAs from HIV+ patients Median age 29 years (IQR 24–35.5)	Inclusion Consecutive HIV+ patients, aged over 16 years, with palpable lymph nodes Exclusion Patients receiving treatment for TB during the previous 3 months	The tissue was homogenised before use	The Xpert MTB/RIF assay was performed following the manufacturer's instructions	AFB microscopy with ZN staining	MGIT 960 and L-J culture for growth detection
Al-Ateah et al. (2012) Kingdom of Saudi Arabia	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=239 specimens from 234 patients Age and HIV status not reported n=172 respiratory: 56 sputum 116 BAL n=67 non-respiratory: 16 tissue biopsies 14 CSF 5 FNA 10 abscess aspirates 13 pleural fluids 3 pericardial fluids 2 synovial fluids 4 abdominal aspirates	Inclusion All clinically suspected TB samples received during the study period Exclusion None	NALC-NaOH processing Tissues and biopsies were ground with a small amount of sterile saline with a tissue grinder and then processed like other specimens	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	AFB smears were prepared, fixed and stained with AUR stain, then visualised with fluorescent microscopy The suspected positive slides were confirmed by ZN stain	L-J medium was inoculated with 0.5 mL of dissolved specimen solution incubated at 37 °C for 8 weeks and examined weekly 0.5 mL was also added to liquid medium in MGITs and incubated in an automated MGIT 960 system™ at 37 °C for 6 weeks
Ani et al. (2009) Nigeria	Level III-2: A comparison with reference standard (not blinded or blinding not	N=40 specimens from 40 children suspected of having TB Age and HIV status not	Inclusion Specimens collected at the Jos University Teaching Hospital	Sputum specimens were decontaminated and centrifuged for sedimentation of	PCR of a 123-base pair target DNA sequence from IS*6110* specific for MTB-complex	ZN AFB microscopy	Duplicate L-J slopes were cultured

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	reported 10 sputum 11 gastric wash 5 CSF 5 ascitic fluid 9 pleural effusions	Jos, Nigeria <u>Exclusion</u> None stated	mycobacteria			
Balcells et al. (2012) Chile	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=160 HIV+ patients Mean age 37.4 years (range 19–65) 81 had two sputum samples 53 had one sputum sample 26 provided mouth wash sample	<u>Inclusion</u> Adults (aged > 18 years) with confirmed HIV infection and suspicion of pulmonary TB They had to have cough (> 10 days), bloody sputum, pneumonia unresponsive to previous antibiotics, fever (> 10 days), abnormal CXR or weight loss <u>Exclusion</u> Empiric anti-TB treatment initiated > 7 days before enrolment	When two sputum samples were collected, a mixture of both was subjected to testing The sputum samples were processed with NALC-NaOH, followed by centrifugation	Xpert MTB/RIF was performed according to manufacturer's instructions Repeated Xpert MTB/RIF assays were performed for patients who had discordant results (AFB-negative with positive Xpert MTB/RIF or vice versa)	Uncontaminated sputum samples and mouthwash were subjected to microscopy with ZN staining	Culture on solid L-J and MGIT liquid medium Cultures were performed for all the samples, irrespective of rapid test results
Bates et al. (2013) Zambia	Level III-2: A comparison with reference standard (not blinded or blinding not	N=930 specimens from children aged 15 years or younger Median age 24 months	<u>Inclusion</u> Any new child inpatient with a primary or secondary	After AFB microscopy sample was taken, samples were homogenised and	The concentrated sample was added to the Xpert MTB/RIF sample reagent in a 1:3 ratio and	Fluorescent AFB microscopy (AUR) was done directly on all samples	1 MGIT tube was inoculated with 0.5 mL concentrated sample and

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	(IQR 12–74) 279 (30%) HIV+ 142 sputum samples 788 gastric aspirates	diagnosis of suspected TB Exclusion Patients who were deemed to have a poor prognosis or if parents or guardians refused consent	digested in NALC-NaOH and concentrated The resulting suspension was used for culture and Xpert MTB/RIF	2 mL of this mixture was added to the Xpert MTB/RIF cartridge and run in the machine in accordance with manufacturer's instructions		incubated in the BACTEC 960 system for up to 42 days DST was done on MTB-positive cultures with the BACTEC MGIT 960 SIRE kit
Baveja et al. (2009) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ? Applicability: C1, P2	N=100 CSF specimens from children strongly suspected of TB meningitis Aged 6 months to 12 years HIV status not reported	Inclusion Children who were presumptively diagnosed with TB meningitis by a set of predetermined criteria Exclusion Patients who were deemed to have a poor prognosis or if parents or guardians refused consent	CSF samples were not pre-treated	PCR amplification was carried out using primers targeting the *MPB64* gene	CSF smear was stained with ZN stain and examined under a microscope	L-J culture and BACTEC medium were inoculated for growth
Ben Kahla et al. (2011) Tunisia	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺	N=333 specimens from 234 patients Age and HIV status not reported n=218 pulmonary (sputum, bronchial wash and gastric lavage) n=115 extra-pulmonary (pleural fluid, joint fluid, pus, cerebrospinal fluid, tissue biopsy,	Inclusion Specimens sent by hospital units to the laboratory for routine diagnosis of TB from December 2007 to September 2008 with sufficient volume to perform all diagnostic tests Exclusion None stated	Pulmonary samples, synovial fluids and pus were liquefied using NALC-NaOH method The remaining samples were directly concentrated by centrifugation	PCR of a 580-bp sequence in the IS*6110* insertion sequence specific for MTB-complex	AFB microscopy was performed from the centrifugation pellets and stained with AUR All positive and doubtful smears were confirmed by ZN technique	Culture was performed onto L-J and/or Coletsos media All isolates were identified to species level using conventional techniques

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Applicability: C1, P2	urine, peritoneal fluid and sperm)					
Bhanothu, Theophilus & Rozati (2014) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P2	N=202 specimens from HIV– patients Mean age 28.5 ± 4.5 years n=123 endometrial tissue biopsies n=68 ovarian tissue biopsies n=11 pelvic aspirated fluids	Inclusion Infertile women highly suspected of having FGTB Exclusion Older than 40 years of age, normal abdominal and vaginal examinations, pregnant and nursing women, severe psychiatric dysfunctions, endocrine problems, sexual disorders, autoimmune disorders, pulmonary or HIV co-infections, diabetes, malnutrition, hypertension, male infertility and ovulation abnormality	Not described	MTB-specific PCR method using primers to detect the *TCR4* gene	AFB smears were stained with ZN stain	Cultures were grown on L-J medium
Bhanu et al. (2005) India	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺	N=18 specimens Aged 20–40 years HIV status not reported 16 endometrial aspirates 14 endometrial biopsies	Inclusion Infertile women with laparoscopic findings suggestive of possible GUTB Exclusion None stated	The samples were decontaminated in NaOH employing modified Hank's flocculation method	PCR of a 123-base pair target DNA sequence from IS*6110* specific for MTB-complex	ZN AFB microscopy	Growth on L-J medium culture was monitored for 8 weeks and the mycobacterial species identified in positive cultures

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Flow and timing ☺ Applicability: C1, P2						
Bhigjee et al. (2007) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=126 CSF specimens from 68 patients Mean age 32.2 ±10 years 48/57 patients were HIV+ HIV status for 11 patients unknown	Inclusion Patients suspected to have neuro TB on clinical grounds They were all AFB-negative	CSF was collected in three consecutive lots of approximately 10 mL: (1) lumbar CSF, (2) thoracic and cervical CSF and (3) CSF at the base of the brain From each specimen of 10 mL of CSF, 5 mL were used for AFB microscopy and culture	PCR and qPCR using primers targeting IS*6110* and the *MPB64* gene	AFB, fluorescent microscopy, after AUR staining	Culture on 7H 11 agar and in mycobacterial indicator growth tubes These specimens were cultured at 37 °C for 6 weeks and examined weekly for growth
Biadglegne et al. (2014) Ethiopia and Germany	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=231 FNA samples from lymph nodes Age and HIV status not reported	Inclusion Patients with enlarged lymph nodes who were not responding to a 2-week course of broad spectrum antibiotics and clinically suspected for TB lymphadenitis Exclusion None stated	Specimens were decontaminated using NALC-NaOH method and centrifuged for sedimentation of mycobacteria	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	AUR AFB microscopy	L-J and Gottsacker slants were inoculated, incubated at 37 °C for 12 weeks and examined weekly BacT/Alert bottles were inoculated, supplemented with antibiotics and then incubated in an automated BacT/Alert 3D System
Carriquiry et al. (2012) Peru	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients	N=131 HIV+ patients (each two sputum samples) Median age 35 years (IQR 29–42)	Inclusion Adults (> 17 years of age) with HIV, and a high suspicion of TB Exclusion	Sputum was decontaminated using NALC-NaOH method	Sample was transferred to the Xpert MTB/RIF cartridge The cartridge was closed and placed into the	Microscopy with ZN staining	Two slopes of L-J culture were inoculated For MGIT, 0.5-mL sputum pellets were

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2		Received > two doses of TB treatment, failure to provide a second sputum sample		GeneXpert System for analysis		inoculated into liquid medium DST was performed using the L-J proportional method
Chakravorty et al. (2006) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=506 sputum samples from 506 patients Age and HIV status not reported	Inclusion: Patients visiting TB centres for the diagnosis of pulmonary TB Exclusion Patients already receiving anti-tubercular treatment	Universal sample processing method, which involves homogenisation and decontamination of specimens by treatment with Universal Sample Processing solution The sample was centrifuged, the sediment was resuspended and then used	PCR assay amplified a 308-bp region of the *devR* gene An additional PCR assay targeting the repetitive IS*6110* sequence was also carried out	USP AFB microscopy with ZN staining	Culture was on L-J slopes Cultures were confirmed to be MTB by the niacin test or by devR PCR
Chakravorty et al. (2005) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ? Applicability: C1, P2	N=571 sputum samples from 571 patients Age and HIV status not reported	Inclusion: Patients with fever, cough, expectoration of sputum, haemoptysis, pain, dyspnoea, weight loss, night sweats, general weakness, positive CXR, mantoux status and any past history of TB Exclusion Receiving anti-	Universal sample processing method (homogenisation and decontamination of specimens by treatment with Universal Sample Processing solution) A subset of 325 samples was also processed by the NALC-NaOH method, centrifuged and the	The isolated DNAs were used for IS*6110*-specific PCRs	USP AFB microscopy with ZN staining	Each sputum sample was decontaminated and inoculated onto L-J medium

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
			tubercular treatment	sediments were used for AFB microscopy and culture			
Davis et al. (2009) Uganda	Level II: A comparison against independent, blinded reference standard among consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☹ Applicability: C1, P2	N=127 sputum samples from 101 outpatients and 26 inpatients Outpatients median age 28 years (IQR 24–35) Inpatients median age 33 years (IQR 28–42) 58/126 (46%) patients were HIV+	Inclusion: Prospectively enrolled outpatients and inpatients aged > 18 years with suspected TB Exclusion Receiving anti-tubercular treatment	Sputum samples obtained from outpatients were processed using dithiothreitol Specimens obtained from inpatients underwent processing using NALC-NaOH Samples were stored frozen	PCR assay targeting the MTB *secA1* gene Two PCRs for each sample were performed in separate capillary tubes	Sputum specimens were examined with direct ZN microscopy on the day of enrolment	Decontaminated sputum was inoculated on L-J media before freezing Frozen samples were cultures using Middlebrook 7H11 agar plates and in MIGT Cultures were considered to be negative if no growth was identified after 8 weeks
de Albuquerque et al. (2014) Brazil	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=140 sputum specimens from 140 HIV+ patients Mean age 37.1 ± 9.9 years	Inclusion Age ≥ 18 years, HIV infected, clinical suspicion of pulmonary TB Exclusion Receiving anti-tubercular treatment, unable to provide sputum samples	Sputum decontamination was undertaken using the NaOH-N-acetyl-L-cysteine method	qPCR: target IS*6110* PCR amplification was performed in triplicate	ZN-stained smears	L-J solid medium and 7H9 broth culture The culture was considered positive when at least one of the media presented mycobacterial growth
Deggim et al. (2013) Switzerland	Level III-2: A comparison with reference standard (not	N=79 mixed specimens Age and HIV status not reported	Inclusion All clinical specimens received to urgently	After Xpert, the remaining sample was decontaminated with	The Xpert MTB/RIF assay was performed following the	AFB microscopy with AUR staining Positive results	MGIT 960 liquid and Middlebrook 7H11 culture media for

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P1	71 respiratory including sputum, BAL 8 non-respiratory including ascetic fluid, pleural fluid, biopsy tissue	confirm or rule out TB in newly identified suspect cases Exclusion None stated	NALC-NaOH and centrifuged for sedimentation of mycobacteria	manufacturer's instructions for respiratory specimens Non-respiratory specimens were tested similarly	were confirmed by ZN staining	growth detection DST was performed using the BACTEC MGIT 960 system
Derese et al. (2012) Ethiopia	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ? Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=134 FNA samples from lymph nodes Mean age 28.6 ± 12.7 years HIV status not reported	Inclusion Retrospective study on previously collected FNA specimens stored at –80 °C to diagnose lymphadenitis TB Exclusion None stated	Specimens were decontaminated using NALC-NaOH method and centrifuged for sedimentation of mycobacteria	PCR was performed using IS*1081* primers	AFB microscopy with ZN staining	Four L-J medium (two with glycerol and two with pyruvate) slopes were incubated and examined weekly for 8 consecutive weeks
Desai et al. (2006) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Patient selection ☺ Index test ☺ Comparator ☺ Reference std ?	N=30 CSF samples Age and HIV status not reported	Inclusion In-house patients with a provisional diagnosis of tuberculous meningitis and had 2 mL of CSF available for study	Sample split in two The first 1-mL portion was centrifuged and used for AFB microscopy and culture, and the second 1-mL portion was stored at −20 °C and used for DNA extraction and PCR	PCR targeting IS*6110* was performed	ZN-stained smears	L-J solid medium

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Flow and timing ☹ Applicability: C1, P2						
Deshmukh et al. (2013) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ? Applicability: C1, P2	N=466 HIV– patients eligible and 463 included in final analysis Mean age 33 ± 21 years n=40 pulmonary: 27 sputum 13 BAL n=423 extrapulmonary: 60 CSF 52 body fluids 164 tissues 94 pus 53 urine	Inclusion Suspected of TB with clinical history available and sufficient volume to perform all diagnostic tests Exclusion If above criteria were not met	The specimens were equally divided into two parts and assigned to the molecular technologist in the molecular diagnostic laboratory for the PCR test, and to the technologist in the mycobacteriology laboratory for AFB microscopy and culture	Specimens from sterile sites were processed first followed by those obtained from non-sterile sites, which were decontaminated using the NALC-NaOH method, in order of AFB scanty PCR was performed using IS*6110* primer sequences	ZN staining	Culture was by both solid medium (L-J) and liquid medium (MGIT) Positive cultures were confirmed for MTB species using the p-nitrobenzoic acid assay
Drouillon et al. (2009) France and Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P1	N=633 specimens (357 from Paris, 100 from Parma and 176 from Rome) Age and HIV status not reported n=548 pulmonary: 417 sputum 46 gastric fluids 68 bronchial aspirates 17 bronchial washes n=59 extrapulmonary: 3 CSF 11 pleural fluids 4 peritoneal fluids 1 pericardial fluid 4 tissue biopsies	A prospective multicentre study that tested both pulmonary and extrapulmonary specimens from patients with suspected TB Inclusion Untreated, at-risk patients who, on the basis of their physicians' initial assessments, were suspected of having active TB Exclusion Patients currently	When necessary, all specimens were decontaminated using the NALC-NaOH procedure	qRT-PCR, which uses the intercalation activating fluorescence DNA probe to emit enhanced fluorescence by binding to a complementary sequence targeting 16S rRNA	AFB microscopy was performed using both AUR and ZN staining	Culture was performed in either liquid (MGIT, BacT/Alert) or solid (L-J and/or Coletsos) medium, with incubation up to 63 days for liquid media and 3 months for solid media at 37 °C Mycobacteria isolated from culture were characterised by molecular assays

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
		12 lymph node punctures 11 urine 5 pus 2 semen 6 stool	receiving anti-TB therapy for more than 6 days or who had completed treatment less than 12 months before the date of enrolment				
Drouillon et al. (2007) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P1	N=179 pulmonary specimens (sputa and gastric fluids) were collected from 100 patients Age and HIV status not reported	Inclusion Consecutive, non-selected patients with suspected TB between April and October 2004 Exclusion None stated	A minimum of 2 mL of pulmonary specimen was collected Some was used directly for the DNA extraction The remainder was decontaminated using NALC-NaOH solution	qPCR was performed to amplify and detect the IS*6110* sequence	Method not specified	Culture was performed using MGIT liquid media and Coletsos slants
Ekrami et al. (2011) Iran	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P2	N=152 sputum samples Age and HIV status not reported	Inclusion Patients who were suspected of having pulmonary TB Exclusion Not reported	Processed according to standard routine diagnostic procedures using the NALC-NaOH method	PCR and nPCR Purified DNA was amplified using primers specific to IS*6110* and two specific pairs of external and internal primers for this bacterium	ZN-stained AFB microscopy	L-J solid medium culture

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
El Khechine et al. (2009) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=134 patients each with one sputum and one stool sample Mean age 37 ± 15 years HIV status not reported	Inclusion Sputum specimens and stool specimens collected from patients suspected of having pulmonary TB Exclusion Not reported	Respiratory tract specimens were digested and decontaminated using the NALC-NaOH method Stool specimens were filtered using a faecal specimen filtration vial kit	qPCR amplification and detection of IS*6110*	Direct ZN-stained microscopy of sputum or filtered stool	Decontaminated sputum was inoculated into a BACTEC 9000 bottle and incubated in an automated BACTEC 9000 MB system for 2 months Stool culture: in L-J medium for 2 months
Ereqat et al. (2011) Palestine	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=95 sputum samples from 84 patients Mean age 46.4 ± 2.5 years HIV status not reported	Inclusion Patients suspected of having pulmonary TB Exclusion Not reported	The sputum samples were processed using the NALC-NaOH method	DNA was extracted from ZN-stained material scraped off from the microscopic slides PCR used primers targeting a 123-bp segment of IS*6110*	ZN-stained AFB microscopy	L-J medium culture (37 °C, up to 8 weeks)
Fan et al. (2014) China	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺	N=200 AFB –ve respiratory samples Mean age 43 ± 18 years 120 sputum 80 BAL	Inclusion Patients suspected of pulmonary TB > 18 years of age with abnormal CXR findings that had three consecutive negative AFB microscopy	Not reported	Simultaneous amplification and testing for MTB (SAT-TB) assay MTB 16S rRNA was reverse transcribed to generate a 170-bp DNA fragment in a real-time PCR	AFB microscopy	MGIT culture was performed in the BD BACTEC MGIT960 Mycobacteria Culture System

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2		results or were sputum scarce <u>Exclusion</u> Patients who were AFB +ve or HIV+ or were missing culture specimens				
George, Mony & Kenneth (2011) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=78 sputum samples Age and HIV status not reported	<u>Inclusion</u> TB suspects <u>Exclusion</u> Not reported	Sputum samples were decontaminated using NALC-NaOH method and stored at –20 °C Decontaminated sputum was processed using the Amplicor respiratory specimen preparation kit	LAMP assay specific for the *rimM* sequence of MTB and *Mycobacterium bovis*	AUR fluorescence microscopy	L-J culture and MGIT culture
Ghaleb, Afifi & El-Gohary (2013) Egypt	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=100 urine samples 75 males with mean age 37.5 ±7.5 years 25 females with mean age 37.0 ± 9.0 years HIV status not reported	<u>Inclusion</u> Patients with symptoms suggestive of renal TB <u>Exclusion</u> None reported	Urine specimens were treated with NALC-NaOH method for the decontamination	PCR targeting IS*6110*	AFB microscopy with ZN staining	L-J solid and BACTEC 12B liquid culture

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Gholoobi et al. (2014) Iran	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=30 clinical samples: 4 urine, 1 gastric washout, 18 BAL, 5 pleural fluid, 1 ascites tap, 1 lung washout) Age and HIV status not reported	Inclusion Specimens from patients suspected of having TB, collected from the Ghaem University Teaching Hospital Exclusion None stated	Each sample was used for three procedures, one for decontamination processing and two (1 mL each) for DNA extraction and PCR	PCR was performed using three sets of specific MTB primers targeting the 16S–23S ITS region, the variable *rpoB* region from MTB and IS*6110*	AFB smear preparation, ZN staining and slide reading were carried out according to the recommendations outlined in the *Manual of TB Bacteriology*	Samples were decontaminated, homogenised and cultured on L-J medium using the Petroff technique
Gomez et al. (2011) Southern Texas (USA, 7%) and Mexico (93%)	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ? Reference std ? Flow and timing ☹ Applicability: C1, P2	N=174 initial participants (136 TB suspects and 38 non-TB controls) 24 were excluded, leaving 150 sputum samples All patients were Hispanics in their mid-40s	Inclusion Patients with suspected pulmonary TB or individuals in whom TB had been ruled out or was unlikely Exclusion Jail inmates, people < 18 years of age, and patients who had received anti-TB treatment for more than 7 days	Sputum was decontaminated using NALC-NaOH and centrifuged, and the pellet was resuspended in a 0.5x final volume of the original sputum	qPCR: targets were IS*6110*, *RD1* and IS*1081*	AFB microscopy (method not recorded)	Decontaminated sample was inoculated in MGIT and L-J media
Haldar et al. (2007) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ?	N=148 sputum samples (selected were direct AFB –ve samples or with low bacterial load) Age and HIV status not reported	Inclusion Subjects attending directly observed treatment short-course centre Patients had negative direct smear or low	USP solution was used: [6 M guanidinium hydrochloride, 50 mM Tris/Cl (pH 7.5), 25 mM EDTA, 0.5% Sarcosyl, 0.1 M ß–	PCR: target genes were *devR* and IS*6110* Two detection formats were employed: molecular-beacon-based end-point detection using the fluorimetric	USP AFB microscopy with ZN staining	Culture: USP-processed deposits were inoculated in 7H9 liquid media containing albumin dextrose complex and PANTA

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Index test ☺ Comparator ☹ Reference std ? Flow and timing ☺ Applicability: C1, P2		bacterial load <u>Exclusion</u> Not reported	Mercaptoethanol]	method, and gel detection using ethidium bromide		(polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin) supplement (Becton Dickinson)
Halse et al. (2010) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P1	N=1,316 specimens for diagnosis of TB Age and HIV status not reported n=1,201 respiratory (sputum, BAL and bronchial wash) n=115 non-respiratory (abscess, aspirates, CSF, gastric fluid, tissue, pleural fluid, wound, liver tissue and lymph node)	<u>Inclusion</u> Clinical specimens received for routine mycobacterial cultivation in the Mycobacteriology Laboratory at the Wadsworth Center, New York State <u>Exclusion</u> Specimens from diagnosed cases of TB	Each respiratory specimen was treated with NALC-NaOH to break up the mucin and to decontaminate the specimens Lung and tissue specimens were ground in disposable tissue grinders until homogeneous, prior to processing	qPCR was performed using IS*6110* and *rpoB* primer sequences qPCR-positive specimens were subjected to pyrosequencing analysis	Smears were prepared by the ZN acid-fast staining method	Processed specimen was inoculated into MGIT tubes and incubated for up to 8 weeks, or until they were found to be positive by the Bactec MGIT 960 instrument L-J slants and Middlebrook selective biplates were also inoculated and incubated at 37 °C, and held for 8 weeks DST for RIF was performed with the MGIT liquid culture
Hanrahan et al. (2014) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ?	N=2,082 individuals had valid culture result (sputum samples) Median age 37 years (IQR 29–46) 58% were HIV+	<u>Inclusion</u> Johannesburg: people aged > 14 years suspected of TB Cape Town: adults aged > 17 years suspected of TB No participants were	Decontamination with NALC-NaOH	Samples were tested using Xpert MTB/RIF G3 cartridge in Johannesburg In Cape Town the second sputum specimen was frozen at −20 °C for later testing using Xpert G2 cartridge	Fluorescence AFB microscopy	Liquid culture using BACTEC MGIT 960

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2		on TB treatment <u>Exclusion</u> Not reported				
Helb et al. (2010) Vietnam	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=107 sputum samples from 107 patients Median age 34 years (range 18–76) 1/107 (0.9%) HIV+	<u>Inclusion</u> Sputum samples from 107 consecutively enrolled patients suspected of having TB	Two sputum samples per patient The first sample was homogenised and split, with some frozen at −70 °C for later analysis by the Xpert MTB/RIF assay and the remainder subjected to AFB microscopy and culture	2–3 mL of digested sputum was transferred to the Xpert MTB/RIF cartridge, the lid was closed, and the cartridge was loaded into the GeneXpert instrument, where all subsequent steps occurred automatically	AFB microscopy	Quantitative culture on L-J medium, and Bactec MGIT 960 liquid culture
Hillemann et al. (2011) Germany	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Reference std ? Flow and timing ☺ Applicability: C1, P1	N=521 non-respiratory specimens Age and HIV status not reported n=91 urine n=30 gastric aspirate n=245 tissue samples n=113 pleural fluid n=19 CSF n=23 stool	<u>Inclusion</u> Consecutive specimens from patients with suspected MTB or NTM infection, not selected by the use of any special criteria <u>Exclusion</u> None	All specimens were processed using the standard NALC-NaOH method	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	Smears were stained by the KCS method and examined with a light microscope	DST for RIF was performed with the MGIT 960 method
Ioannidis et al. (2011) Greece	Level III-2: A comparison with reference standard (not blinded or blinding not	N=105 AFB –ve pulmonary and extrapulmonary samples Age and HIV status not	<u>Inclusion</u> Specimens were selected from patients with strong clinical	All specimens were processed using the standard NALC-NaOH method	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the	AFB smears of the processed specimens were prepared and examined	Solid (L-J) and liquid (MIGT 960) culture media were inoculated RIF resistance of

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	reported	indications for TB <u>Exclusion</u> None		GeneXpert instrument		bacterial colonies were investigated with the GenoType MTBDRplus assay and confirmed by DST using the proportion method on L-J culture medium and/or MGIT for RIF
Jiang et al. (2012) China	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=235 mixed specimens: sputum (88.1%), pleural fluid (3.0%), lymph node (3.0%), CSF (2.1%), urine (1.7%), abscess and exudate (1.7%) and faeces (0.4%) Age and HIV status not reported	<u>Inclusion</u> Clinical specimens were obtained from patients with suspected TB <u>Exclusion</u> None	Respiratory specimens were decontaminated with NALC-NaOH Extrapulmonary specimens from closed and normally sterile sites were used directly without decontamination after a single centrifugation	qRT-PCR using MTB 16S rRNA-specific primers	AFB smears with ZN stain	Liquid MGIT 960 and solid L-J cultures
Keys et al. (2012) Australia	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ? Index test ? Comparator ? Reference std ? Flow and timing ?	N=6 pleural biopsied from children Age and HIV status not reported	<u>Inclusion</u> Children with clinical suspicion of TB with both respiratory and constitutional symptoms <u>Exclusion</u> None stated	Not reported	PCR, details not provided	AFB microscopy	Culture

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Applicability: C1, P1						
Khan, Cheema & Khan (2013) Egypt	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ? Reference std ? Flow and timing ? Applicability: C1, P2	N=50 urine samples Median age of patients 38 years (range 20–76) HIV status not reported	Inclusion Patients with symptoms suggestive of GUTB Exclusion None reported	Urine specimens were treated using NALC-NaOH method for the decontamination	PCR, details not provided	AFB microscopy with ZN staining	Culture on L-J medium
Khosravi et al. (2010) Iran	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=200 urine samples Mean age 37.8 years HIV status not reported	Inclusion Patients with symptoms suggestive of renal TB Exclusion None reported	Three urine samples collected on three consecutive days as early morning urine, pooled and concentrated	nPCR targeting IS*6110*	AFB microscopy with ZN staining	Culture on L-J medium with a conventional identification procedure
Kibiki et al. (2007) Tanzania	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias	N=120 BAL samples from 120 HIV+ patients Mean age 39 years	Inclusion HIV+ patients aged > 17 years with features of chest infection and referred for bronchoscopy > 80% had previous	BAL samples were pre-treated by decontamination with NaOH and centrifuged The sediment was used for the different	PCR (40 cycles) targeting IS*6110*	Direct smears were examined for AFB after ZN staining	MTB culture was performed using in-house L-J solid medium, with a maximum incubation period of 8 weeks

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2		antibiotic treatment for pneumonia <u>Exclusion</u> Pregnant women and patients with oxygen saturation < 90% under 6 L/minute	diagnostic tests			
Kim et al. (2008) Korea	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=2,973 patients Age and HIV status not reported n=1,134 pulmonary specimens: 863 sputum 271 bronchial aspirate n=1,839 extrapulmonary specimens: 834 pleural fluid 313 CSF 248 urine 147 tissue 109 pus 59 peritoneal fluid 34 blood 12 gastric aspirate 9 pericardial fluid 7 bone marrow 67 other	<u>Inclusion</u> Patients who visited Kyung Hee Medical Center between July 2003 and July 2006 for TB diagnosis <u>Exclusion</u> None	Sputum, bronchial aspirate, urine and pus were incubated with NaOH and then centrifuged Tissues were minced with scissors and treated with proteinase K and then centrifuged Cerebrospinal and other body fluids were centrifuged without any pre-treatment	nPCR using primers targeting IS*6110*	AUR-stain positive specimens were confirmed with ZN microscopy	Specimens were inoculated onto 3% Ogawa media and then incubated for at least 8 weeks at 37 °C
Kurbatova et al. (2013) Russia	Level III-2: A comparison with reference standard (not blinded or blinding not known)	N=238 sputum specimens from 201 patients Age and HIV status not reported	<u>Inclusion</u> Adults (> 17 years of age) with presumptive or recently diagnosed pulmonary TB	Sputum samples were homogenised and split into two portions. From one portion, 1.0 mL was tested by Xpert	The Xpert MTB/RIF assay was performed according to the manufacturer's instructions	Direct and AUR fluorescence microscopy	Sample was inoculated onto L-J solid medium and BACTEC MGIT 960 liquid medium

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Quality: Low risk of bias Patient selection ? Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2		Exclusion Receiving anti-TB drugs within 60 days prior to specimen collection	and a smear prepared for ZN microscopy The remaining portion (≥ 3 mL) was decontaminated with NALC-NaOH and centrifuged for culture and Xpert	The results were obtained using the Xpert MTB/RIF software		Culture-based DST was performed using either the BACTEC MGIT 960 system or the absolute concentration method on L-J medium
Lee et al. (2013) Korea	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=35 culture-positive Xpert-positive bronchoscopy samples Age and HIV status not reported	Inclusion Retrospective review of all records for patients with suspected PTB, among whom the AFB microscopy, culture and Xpert assays were performed using bronchial washings or BAL Exclusion Patients diagnosed with sputum AFB +ve PTB before bronchoscopy or who had received anti-TB medication for ≥ 2 weeks within 90 days before bronchoscopy	Samples were decontaminated with NaOH and centrifuged for AFB microscopy, culture and Xpert	The Xpert MTB/RIF assay was performed following the manufacturer's instructions	The AFB smears were examined after AUR staining	Culture-based DST was performed using the proportion method on 3% Ogawa medium
Lee, Chen & Peng (2009) Taiwan	Level III-2: A comparison with reference standard (not blinded or blinding not known)	N=150 sputum specimens Age and HIV status not reported	Inclusion Suspected TB patients admitted to Kaohsiung Medical University Hospital	Decontamination using NALC-NaOH treatment, and subsequent concentration by	LAMP assay for detection of 16S rRNA in clinical isolates of MTB using an ELISA detection system	AFB staining (method not specified)	Mycobacterial culture (method not specified)

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ? Applicability: C1, P2		Exclusion Not reported	centrifugation			
Ligthelm et al. (2011) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=48 lymph node FNAs Mean age of patients 27.9 ± 15.1 years 36/48 had unknown HIV status 9/12 (75%) patients tested were HIV+	Inclusion All patients referred for FNA biopsy with possible TB lymphadenitis Exclusion Inadequate sample for testing	Sample used directly.	The Xpert MTB/RIF assay was performed following the manufacturer's instructions	AFB smears with both ZN staining and fluorescence microscopy	MGIT 960 for growth detection
Makeshkumar, Madhavan & Narayanan (2014) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=178 extrapulmonary specimens Age and HIV status not reported 59 ascetic fluid 54 pleural fluid 25 CSF 12 FNA 8 urine 7 pus 6 synovial fluid 7 other	Inclusion All clinically suspected extrapulmonary TB patients who were visiting SRM Medical College Hospital during the period May 2008 – May 2009 Exclusion None	Sterile body fluid samples (ascitic fluid, pleural fluid, CSF, synovial fluid, pericardial fluid and pancreatic cyst fluid) were centrifuged Pus specimens were decontaminated using Petroff's method Biopsy and skin tissue samples were ground and then centrifuged	PCR using primers targeting IS*6110*	AFB microscopy with ZN stain	Specimens were inoculated onto solid L-J medium and examined every second day during the first week and weekly for up to 8 weeks

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Malbruny et al. (2011) France	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=180 specimens from 132 patients Age and HIV status not reported N=91 respiratory: 18 sputum 31 bronchial aspirate 9 BAL 33 gastric aspirate N=89 non-respiratory: 15 CSF 23 lymph node 6 vertebral biopsy 5 joint fluid 12 pleural fluid 3 peritoneal fluid 3 urine 22 other	Inclusion Specimens from patients clinically suspected of TB were prospectively collected Exclusion None	All respiratory samples were digested and decontaminated using NALC-NaOH, whereas most of the non-respiratory samples were not All biopsy samples were processed using a homogeniser All samples except CSF were concentrated by centrifugation	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	Fixed preparations were stained with AUR and visualised under a fluorescence microscope	Liquid medium (MGIT) and Coletsos slants were inoculated Liquid cultures were monitored by the automated MGIT 960 system for up to 6 weeks, while solid media were kept for up to 12 weeks Positive cultures were confirmed using a commercial immuno-chromatographic assay
Marchi et al. (2008) Brazil	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=117 sputum specimens Age and HIV status not reported	Inclusion Suspected TB patients whose sputum samples were sent to the Municipal Public Laboratory for *Mycobacterium* spp. testing Exclusion Not reported	For culture and PCR, samples were treated with NaOH and SDS, followed by neutralisation with phosphoric acid	PCR was carried out using primers specific for 123-bp product of IS*6110*	ZN staining was carried out directly on sputum sample smears	Culture of the treated samples was carried out in L-J-MTBAC culture media and incubated at 37 ºC for 8 weeks
Marlowe et al. (2011) USA	Level III-2: A comparison with reference standard (not	N=217 respiratory specimens (126 AFB +ve and	Inclusion Specimens ordered at three different sites in	The NALC-NaOH method was used to digest, decontaminate	The treated specimen sample was transferred to the Xpert MTB/RIF	A smear of the processed sediment was	Culture method not described DST was performed

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P1	91 AFB –ve) Age and HIV status not reported	western USA for routine mycobacterial testing were included in the study <u>Exclusion</u> None stated	and concentrate respiratory specimens	cartridge and the test was run in the GeneXpert instrument	prepared, stained and read Method not stated	by a broth micro-dilution method
Mashta et al. (2011) India	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2	N=463 sputum samples Age and HIV status not reported	<u>Inclusion</u> Samples from patients suspected of TB <u>Exclusion</u> Not reported	Sputum samples used directly for AFB microscopy and culture at NDTB centre Transferred cool to NII, where samples were sputum liquefaction and decontamination occurred	PCR, targeting IS*6110* and *devR*	AFB smear: staining by free carbol fuchsin, decolourising by 25% sulphuric acid and counterstained by 0.1% methylene blue	L-J medium culture Samples were liquefied by 4% NaOH solution for 20 minutes, centrifuged at 3,000 g, and pellet was washed twice with distilled water
Maurya et al. (2011a) India	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☹	N=102 pleural effusions from 102 patients Mean age 30.4 ± 13.2 years 2/102 (2%) were HIV+	<u>Inclusion</u> Clinically suspected cases of pleural TB <u>Exclusion</u> Patients with undetermined aetiology	Specimens were divided into two parts and kept at −20 °C until processing (method not described)	PCR was performed using IS*6110* primer sequences	Smear examination was by ZN staining	BACTEC vials were incubated and interpreted as per the Becton Dickinson instruction manual

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Applicability: C1, P2						
Maurya et al. (2011b) India	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2	N=328 extrapulmonary specimens from new cases suspected of TB Mean patient age 39.8 ± 16.1 years HIV status not reported Lymph node aspirates, cold abscesses, pleural fluid, CSF, synovial fluid, ascetic fluid, urine, gastric aspirate, pus, bone marrow, wound and pus swabs, and biopsy tissues	Inclusion Non-repeated specimens from suspected cases of extrapulmonary TB Exclusion None stated	Specimens were divided into two parts and kept at −20 °C until processing (method not described)	PCR was performed using IS*6110* primer sequences	Smear examination was by ZN staining	BACTEC vials were incubated and interpreted as per the Becton Dickinson instruction manual
Michelon et al. (2011) Brazil	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=476 sputum specimens Age and HIV status not reported 301 induced sputum specimens 175 spontaneous sputum specimens: 47 patients with a single collection that were processed in duplicate 128 patients with two collections on different days that were processed at a single time	Inclusion Samples of suspected TB patients Exclusion Not reported	All samples were treated with NALC-NaOH Mycobacterial culture and AFB microscopy were carried out for all clinical samples and the association of these results was chosen as the gold standard	PCR amplification reactions were performed with biotinylated primers derived from IS*6110* This was followed by a microwell hybridisation assay to detect the amplified product	Method not reported	Method not reported

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Min et al. (2010) Korea	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=136 bronchial aspirates Age range 17–88 years HIV status not reported	Inclusion Consecutive patients suspected of TB who did not produce sputum or who produced AFB-negative sputum and underwent RT-PCR in bronchial aspirate for the diagnosis of TB Exclusion HIV+ and immunocompromised patients	Bronchial aspirate was digested and decontaminated with NALC-NaOH	qPCR was performed using primers targeting the *senX3-regX3* intergenic region This was designed to be positive for MTB and negative for *Mycobacterium bovis* bacille Calmette-Guérin	AFB microscopy	Mycobacteria were cultured on 3% Ogawa media for a maximum period of 8 weeks
Mittal et al. (2011) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=50 lymph node FNAs Mean age of patients 27.9 ± 15.1 years HIV status not reported	Inclusion Patients with peripheral lymphadenopathy clinically suspected to be of TB origin Exclusion Patients with clinically non-palpable lymph nodes, those already on anti-TB treatment and those who had a known malignancy	The material was used directly for ZN staining and culture, and a portion was frozen at −20 °C for use in a PCR	PCR was performed using IS*6110* primers	AFB microscopy with ZN staining	Cultures were inoculated on L-J medium and incubated at 37 °C The L-J slants were examined weekly for any growth for 8 weeks
Moure, Martin & Alcaide (2012) Spain	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias	N=149 AFB-negative extrapulmonary specimens Age and HIV status not reported 14 CSF 31 pleural fluid	Inclusion AFB –ve samples (one sample per patient) collected from July 1999 to May 2011 in Costa Ponent Exclusion	Non-sterile clinical samples were pre-treated using the NALC-NaOH digestion-decontamination method	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	Microscopic examination with AUR and ZN stains	Mycobacterial culture using L-J and MGIT mediums Positive cultures were confirmed as MTBC by the use of DNA probes

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P1	7 joint fluid 3 ascitic fluid 3 pericardial fluid 8 gastric aspirates 4 urine 38 FNA (lymph nodes) 19 abscess aspirates 20 tissue biopsies 2 stool	None	Sterile fluid specimens were directly processed, and biopsy specimens were disaggregated with a mortar and then resuspended			
Nakiyingi et al. (2012) Uganda	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Some risk of bias Patient selection ☺ Index test ☺ Comparator: ☺ Reference std ☺ Flow and timing ? Applicability: C1, P2	N= 205 patients with AFB –ve sputum samples Mean age 34.7 ± 10.4 years 176/205 (86%) were HIV+	Inclusion TB suspects with cough for ≥ 2 weeks with/without sputum production, who had other signs of TB Exclusion AFB-positive patients	Sputum was decontaminated using NALC-NaOH and centrifuged	PCR, targeting IS*6110*	AFB microscopy using ZN staining	Sputum was inoculated into L-J culture bottles and incubated at 37 °C for up to 3 months
Nicol et al. (2011) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=452 children with at least one induced sputum specimen Median age 19.4 months (IQR 11–46) 108/452 (24%) were HIV+	Inclusion Consecutive children, aged 15 years or younger, admitted to hospital with suspected pulmonary TB on the basis of having a cough for more than 14 days plus one more sign suggestive of pulmonary TB Exclusion	Sputum specimens were processed within 2 hours of collection in an accredited routine diagnostic microbiology laboratory by trained technicians who used standardised NALC-NaOH protocols	The concentrated sample was added to the Xpert MTB/RIF sample reagent in a 1:3 ratio, and 2 mL of this mixture was added to the Xpert MTB/RIF cartridge and run in the machine in accordance with manufacturer's instructions	A drop of sediment was used for fluorescent AFB microscopy	Automated liquid MGIT culture was done with 0.5 mL of the resuspended pellet Cultures were incubated for 6 weeks if negative

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
			More than 72 hours of TB treatment, could not be followed up, no informed consent, or if an induced sputum specimen could not be obtained				
Pahwa et al. (2005) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☹ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=100 FNAs from 100 patients 55 had PCR results Age range 2.5 months – 60 years HIV status not reported	Inclusion Patients with clinically and cytologically suspected TB lymphadenitis The clinical symptoms suggestive of TB were fever, anorexia or weight loss, and lymphadenopathy	FNAs from the involved lymph node were divided into seven parts; five were used for AFB stains, one for PCR and one for L-J medium culture	PCR was performed on all specimens, targeting the gene encoding the MPB64 protein	AFB using ZN and fluorescent staining	L-J medium culture FNAs were liquefied and digested using N-acetyl L-cysteine, decontaminated by standard procedure using Petroff's method, inoculated on to L-J slants and incubated at 37 ºC for 6–8 weeks
Park et al. (2013) Korea	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2	N=320 respiratory specimens from 311 adult patients Age and HIV status not reported 254 sputum 66 BAL	Inclusion Samples were prospectively collected from patients with suspected pulmonary TB between 26 May 2011 and 2 December 2011 at a tertiary care hospital Exclusion None stated	The respiratory specimens were processed with NALC-NaOH, followed by centrifugation For the Xpert assay, 1 mL of a respiratory specimen without decontamination or concentration was used	The specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	Specimens were examined blindly by fluorescence staining	Specimens were examined by cultures with both solid and liquid media

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Rachow et al. (2011) Tanzania	Level II: A comparison against independent, blinded reference standard among consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☹ Applicability: C1, P2	N=292 patients with sputum samples (each provided three samples, results were recorded from one—the morning sample) Mean age 39.2 ± 13.8 years 58.9% were HIV+	Inclusion Patients with symptoms suggestive of pulmonary TB Exclusion Not able to produce sputum at recruitment, lost to follow-up during recruitment procedures	Sputum samples were split into two aliquots, one of which was stored at –80 ºC; the other was processed for standard AFB microscopy and culture Sputa were decontaminated using the NALC-NaOH method	Frozen sputa were thawed and processed according to the Xpert MTB/RIF assay test procedure	AFB microscopy with ZN staining	L-J solid and MGIT liquid culture
Santos et al. (2006) Brazil	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=218 sputum samples 60 non-Indigenous 158 Indigenous Age and HIV status not reported	Inclusion Non-Indigenous and Indigenous patients presenting with respiratory symptoms and suspected of pulmonary TB, providing sputum sample Exclusion Not reported	48 samples had to be transported in cetylpyridinium chloride solution and were then homogenised	PCR targeting IS*6110*	AFB microscopy using direct and concentrated techniques 48 samples were stained with bromothymol blue	L-J medium culture (using NaOH as decontaminant)
Sharma et al. (2012) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ?	N=80 osteoarticular TB (OATB) specimens 67 synovial fluid 13 pus Age and HIV status not reported	Inclusion Specimens received for AFB staining and culture for diagnosis of OATB Exclusion None stated	The specimens were concentrated by centrifugation	M-PCR with primers targeting IS*6110* and *MPB64*	AFB microscopy with ZN staining	Culture was on L-J medium

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2						
Sharma et al. (2013) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=50 endoscopic ileocaecal biopsies Age range 19–68 years HIV status not reported	Inclusion Endoscopic ileocaecal biopsy received for acid-fast staining and culture were tested from December 2008 to March 2010 Exclusion None stated	Samples were decontaminated and concentrated using NALC-NaOH method The samples were centrifuged and the sediment was resuspended and prepared for AFB microscopy, culture and M-PCR	M-PCR using primers specific for IS*6110* and *MPB64*	ZN AFB microscopy	Culture was done on two L-J slants using standard procedures and incubated for 6 weeks
Shukla et al. (2011) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=140 clinical samples from patients mostly 21–30 years of age HIV status not reported N=86 pulmonary: 74 sputa 12 gastric aspirates N=54 extrapulmonary: 16 CSF 38 endometrial biopsies	Inclusion Patients attending outpatient and inpatient departments were selected for this study on the basis of radiological diagnosis and other investigations Exclusion None	The sputum was digested and decontaminated using NALC-NaOH method, and concentrated by centrifugation Biopsy tissues were first ground in a sterile mortar and pestle, then decontaminated CSF was used directly	A two-step nPCR to amplify a 123-bp DNA segment belonging to IS*6110*	Direct microscopic examination using ZN method	L-J slants were inoculated, then incubated at 37 °C for 6–8 weeks
Singh et al. (2006) India	Level III-1: A comparison against independent, blinded	N=85 bone-marrow aspirates Age and HIV status not	Inclusion Patients who had fever of unknown	The samples were decontaminated using NALC-NaOH	PCR targeting the *MPT64* gene of MTB	AFB smears were prepared using ZN stain	Culture was on duplicate L-J slopes, and growth was monitored for

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ☺ Flow and timing ☺ Applicability: C1, P2	reported	origin alone or associated with cervical lymphadenopathy, ascites, bone marrow transplant, as well as those with pyrexia accompanying renal failure or aplastic anaemia were investigated for TB <u>Exclusion</u> None	processing			8 weeks
Sohn et al. (2014) Canada	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P1	N=436 sputum samples Median age of patients 44 years (IQR 31–61) 12/49 (24%) patients tested were HIV+	<u>Inclusion</u> Consecutive patients aged > 17 years, referred for evaluation of suspected active pulmonary TB <u>Exclusion</u> Not reported	Not reported	The Xpert MTB/RIF was performed at the TB clinic according to the standard protocol for unprocessed samples, per the manufacturer	AFB microscopy with AUR staining	Liquid culture on three processed samples was followed by phenotypic culture-based DST at the provincial reference laboratory
Suzuki et al. (2006) Japan	Level II: A comparison against independent, blinded reference standard among consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ?	N=138 sputum specimens Age and HIV status not reported	<u>Inclusion</u> Patients hospitalised in Minami–Yokohama National Hospital, under suspicion of TB during a designed 2-month period <u>Exclusion</u>	The clinical specimens were treated with Sputerzyme and then decontaminated using the NALC-NaOH method	PCR–ICA DNA amplification with labelled primers targeting d*naJ* and using immune-chromatographic detection of the amplified product by application on a sample pad of the test	Specimens were fixed and stained with AUR, and their fluorescence was examined using microscopy The presence of AFBs was	Specimens were inoculated into MGIT 960 tubes Growth of MTB was evaluated on the consumption of oxygen in the medium, as

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2		Not reported		strip	confirmed by ZN staining	monitored using the MGIT 960 system
Teo et al. (2011) Singapore	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=162 non-duplicated clinical specimens Age and HIV status not reported N=131 respiratory: 124 sputum 5 BAL 2 tracheal aspirate N=31 non-respiratory: 5 gastric aspirates 3 urine samples 7 CSF 5 body fluids (pleural, pericardial, ascites) 10 miscellaneous such as pus and biopsies	Inclusion Patients attending outpatient and inpatient departments were selected for this study on the basis of radiological diagnosis and other investigations Exclusion None	Specimens of a fluid nature were decontaminated according to standard methods using NALC-NaOH Tissue specimens were thoroughly minced using a pair of sterile scissors before being used For normally sterile body fluids, decontamination was not performed Specimens were then concentrated by centrifugation	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	Direct microscopic examination using ZN method	MGIT tubes were inoculated with 0.5 mL of the processed specimen and then incubated in the MGIT 960 instrument at 37 °C L-J slants were inoculated and then incubated at 37 °C for 6–8 weeks
Therese, Jayanthi & Madhavan (2005) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☺	N=280 extrapulmonary clinical samples Age and HIV status not reported 104 peritoneal fluids 3 pericardial fluid 120 CSF 44 lymph node FNA 9 tissue biopsies	Inclusion Specimens from patients who were clinically and/or radiologically diagnosed as having TB Exclusion None	Aspirated fluid specimens such as ascitic fluid and cerebrospinal fluid were concentrated by centrifugation Tissue specimens were cut into tiny pieces with sharp scissors and homogenised in a glass tissue grinder	nPCR using primers targeting for MPB64 protein	AFB smears were stained by ZN method	Cultures were on L-J medium in duplicate

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Applicability: C1, P2			and used directly			
Theron et al. (2013) South Africa	Level III-2: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=156 patients with BAL samples Median age 46.1 years (IQR 33.1–55.7) 46/156 (35%) were HIV+	Inclusion Patients > 17 years of age with suspected pulmonary TB who were referred for bronchoscopy Exclusion Patients on anti-TB treatment, contaminated culture	BAL fluid was split and one aliquot was decontaminated by NALC-NaOH and examined by microscopy and culture; the second aliquot was used for Xpert NAAT	The Xpert MTB/RIF assay was performed on 1 mL of BAL fluid and, when available, a median volume of 10 mL was concentrated and resuspended in 1 mL of sterile phosphate-buffered saline	Fluorescence AFB microscopy	Liquid culture for MTB using the BACTEC MGIT 960 system Culture-positive isolates underwent routine phenotypic DST for rifampicin and isoniazid using the MGIT 960 SIRE kit
Theron et al. (2012) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=480 patients (each had two sputum samples) Age and HIV status not reported	Consecutive patients with suspected TB, who provided two sputum samples, and provided informed consent Exclusion Not reported	Not reported	2–3 mL of digested sputum was transferred to the Xpert MTB/RIF cartridge, the lid was closed, and the cartridge was loaded into the GeneXpert instrument, where all subsequent steps occurred automatically	Concentrated fluorescent AFB microscopy	Culture using BACTEC MGIT 960 medium
Tortoli et al. (2012) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias	N=1,493 extrapulmonary samples corresponding to 1,068 patients Age and HIV status not reported 330 pleural fluids	Inclusion Retrospective results from consecutive extrapulmonary specimens accepted by eight Italian laboratories for the	Non-sterile samples were decontaminated using standard NALC-NaOH procedure and concentrated by centrifugation Sterile samples were	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and test was run in the GeneXpert instrument	AFB microscopy used AUR staining	Culture was in both solid (L-J) and liquid (MGIT) media

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	224 gastric aspirates 195 pus 133 CSF 130 urine 94 cavity fluids 368 tissue biopsies	diagnosis of EP0-TB Exclusion None	mechanically homogenised (if needed) before concentration			
Vadwai et al. (2011) India	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=547 extrapulmonary specimens from 547 patients Median age 37 years (range 8 months – 94 years) HIV status not reported 284 biopsy specimens (147 from tissues, 82 from lymph nodes and 55 FNAs) 147 pus 93 body fluids (11 synovial, 3 pericardial, 66 pleural and 13 peritoneal) 23 CSF	Inclusion Samples from consecutive patients suspected of extrapulmonary TB in a private tertiary care hospital if they could provide detailed clinical history and radiological and histology/cytology reports, and an adequate amount of specimen material Exclusion None reported	The sample was divided equally into three parts One part was processed with NALC-NaOH and centrifuged prior to culture	A 2:1 volume of sample reagent buffer was added to biopsy specimens after they had been chopped into very small pieces with a sterile blade in a sterile petri dish prior to adding to the cartridge The Xpert MTB/RIF test was run in the GeneXpert instrument	Direct and concentrated AFB microscopy with ZN staining	L-J medium and liquid medium (MGIT) culture-positive results were confirmed for MTB by a p-nitrobenzoic acid assay and subjected to indirect DST with MGIT SIRE
Van Rie et al. (2013b) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ?	N=361 HIV+ patients with two lymph node FNA samples Mean age 35.8 years (range 18–73)	Inclusion HIV+ patients clinically suspected of having lymph node TB, age > 17 years, not receiving treatment for active or latent TB	The first FNA was smeared on two slides and fixed for cytology and AFB ZN microscopy The second FNA was smeared on a slide and air-dried for AUR staining	Xpert MTB/RIF 1 mL of the needle washing liquid was mixed with 2 mL of the Xpert sample reagent buffer	AFB microscopy with AUR staining	The remainder of the needle washing saline solution for Xpert was sent for processing and inoculation into a MGIT culture medium

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
	Flow and timing ☺ Applicability: C1, P2						
Walusimbi et al. (2013) Uganda	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator: ☺ Reference std ☺ Flow and timing ☹ Applicability: C1, P2	N=430 AFB –ve, HIV+ sputum samples Median age 34 years (IQR 29–40) 369 had valid culture and Xpert results	Inclusion HIV+ patients with symptoms of TB, giving consent, providing spot and early-morning sputum sample Exclusion Patients on TB treatment or unable to produce sputum	The samples were digested and decontaminated using the NALC-NaOH method, and then concentrated by centrifugation	For the Xpert MTB/RIF assay, a sample reagent was added to the processed sample in a 3:1 ratio The mixture was introduced into a cartridge, which was then loaded into the GeneXpert instrument, where the test was performed automatically When sufficient residual was available, repeat testing was carried out when an 'invalid' or 'error' result was obtained	Fluorescent microscopy (unprocessed) using standard AUR reagent	Culture using MGIT and L-J medium
Zar et al. (2013) South Africa	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=384 children with induced sputum samples Median age 38.3 months (IQR 21.2–56.5) 31/384 (8%) were HIV+	Inclusion Consecutive children < 15 years of age presenting from 1 August 2010 to 30 July 2012 with suspected pulmonary TB Exclusion None reported	Samples were decontaminated using standard NALC-NaOH procedure and concentrated by centrifugation	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and test was run in the GeneXpert instrument	Fluorescent AFB microscopy using AUR	MGIT culture was done using 0.5 mL of resuspended pellet on sputum specimens and incubated for up to 6 weeks

Study Country	Study design Quality appraisal	Study population	Inclusion criteria / exclusion criteria	Sample preparation	Intervention	Comparator	Reference standard
Zeka, Tasbakan & Cavusoglu (2011) Turkey	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☺ Index test ? Comparator ☺ Reference std ? Flow and timing ☹ Applicability: C1, P2	N=429 specimens from 429 patients Median age 47.5 ± 22.2 years HIV status not reported N=253 pulmonary (sputum, BAL, bronchial aspirate and gastric fluid specimens) N=176 extrapulmonary (pleural fluid, lymph node biopsy, disc material, ascitic fluid, cerebrospinal fluid, pericardial fluid, skin biopsy and urine specimens)	Inclusion Samples from patients suspected of TB sent to the Department of Medical Microbiology, Mycobacteriology Laboratory between February 2010 and November 2010 Exclusion None	Non-sterile clinical specimens were processed using the conventional NALC-NaOH method	The treated specimen sample was transferred to the Xpert MTB/RIF cartridge and the test was run in the GeneXpert instrument	After decontamination, smears were prepared by the AUR acid-fast staining method	Decontaminated specimens were inoculated to L-J solid medium and MB/BacT liquid medium for growth detection DST was performed on the first positive culture from each specimen using the proportional method with 7H10 agar medium and confirmed by the GenoType MTBDR plus assay

AUR = auramine-based fluorochrome; BAL = bronchoalveolar lavage; CSF = cerebrospinal fluid; CT = computed tomography; DST = drug susceptibility testing; FGTB = female genital tuberculosis; FNA = fine-needle aspirate; GUTB = genitourinary tract TB; HIV = human immunodeficiency virus; KCS = Kinyoun cold staining; LAMP = loop-mediated isothermal amplification; L-J = Lowenstein-Jensen; MGIT = Mycobacterium Growth Indicator Tubes; M-PCR = multiplex PCR; MTB = *Mycobacterium tuberculosis*; NALC-NaOH = N-acetyl-L-cysteine and sodium hydroxide; nPCR = nested PCR; OATB = osteoarticular TB; PCR = polymerase chain reaction; PCR-ICA = PCR-immunochromatographic assay; qPCR = quantitative (real-time) PCR; RT-PCR = reverse transcription PCR; TB = tuberculosis; USP = universal sample processing; ZN = Ziehl-Neelsen

Table 97 Study profiles of included studies providing linked evidence on the change in management following NAAT on patients suspected of having TB

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Boehme et al. (2010) Foundation for Innovative New Diagnostics, Geneva, Switzerland Conducted at: Urban health centres in: Lima (Peru), Baku (Azerbaijan), Cape Town (South Africa), Kampala (Uganda), Vellore (India), Manila (Philippines)	Historical control study Level: III-3 Quality: 18/26 Some risk of bias	N=6,648 (5,862 suspected of TB, 786 suspected of MDR-TB) Median age: 38 years (IQR 29–50) 2,605 (39%) females 1,255 (19%) HIV infected 3,509 (53%) HIV status unknown	Inclusion: Adults aged > 17 years with > 2 weeks of cough, provided at least two sputum samples Exclusion: Second sputum sample was collected > 1 week from the first, no (valid) culture conducted, no valid MTB/RIF result, AFB-positive with no positive culture, only one positive culture with 20 or fewer colonies for solid culture or more than 28 days to positivity for liquid culture, a positive culture during follow-up only, only one positive culture with missing speciation result, a positive culture with NTM growth, or discrepant RIF results by conventional drug susceptibility testing in two samples	Xpert MTB/RIF assay Routine AFB microscopy, and culture	Same tests, but in comparator group Xpert results were not reported to clinicians or used for patient management	Proportion of results reported to the clinics for each method from date of first sputum sample Time to TB detection (by each method) Time to treatment
Buchelli Ramirez et al. (2014) Hospital Universitario Central de Asturias, Oviedo, Spain Conducted at: Hospital Universitario Central de Asturias, Oviedo, Spain	Retrospective cohort study Level: III-3 Quality: 19.5/26 Some risk of bias	N=128 patients Mean age 52 ± 23 years 43 (33.6%) females	Inclusion All patients diagnosed with pulmonary TB between January 2010 and July 2012, including cases with bronchial confirmation alone Exclusion: Not reported	Xpert MTB/RIF, AFB microscopy and mycobacterial culture	NA	CIM: time to treatment System-related treatment delay
Davis et al. (2014) San Francisco General Hospital, University of California, San Francisco, USA	Prospective cohort Level: III-3 Quality: 17/26 Some risk of bias	N=227/538 included, but only 156 were tested by NAAT Median age: 52 years (IQR 39–60) 54 (35%) females	Inclusion: Consecutive adults undergoing evaluation for active pulmonary TB at the San Francisco Department of Public Health TB clinic between May 2010 and	Xpert MTB/RIF on sputum specimen, AFB microscopy and culture for MTB	(Empiric treatment decision pending other test results)	Unnecessary treatment rate

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Conducted at: San Francisco Department of Public Health		13 (8%) HIV infected Two key groups of patients for Xpert NAAT: (1) those initiating empiric treatment for active TB and (2) those coming from congregate settings (e.g. homeless shelters, behavioural treatment programs, dialysis centres)	June 2011 Exclusion: Patients with incomplete microbiologic or clinical follow-up data, reporting TB treatment at time of Xpert NAAT			
Fan et al. (2014) Tuberculosis center for diagnosis and treatment, Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China Conducted at: Shanghai Pulmonary Hospital, Tongji University School of Medicine, Shanghai, China	Prospective cohort Level: III-3 Quality: 17.5/26 Some risk of bias	N=280/335 included Mean age: 43 ± 18 years 54 (25%) females	Inclusion: Patients with abnormal chest radiographic findings compatible with active TB (TB suspects), > 18 years of age, sputum scarce or with negative AFB microscopy Exclusion: AFB-positive patients and HIV positive patients	SAT-TB assay (in-house) and culture (liquid medium)	NA	CIM: time to detection of TB
Guerra et al. (2007) School of Medicine, Johns Hopkins University, Baltimore, MD, USA Conducted at: Baltimore City Health Department, USA	Historical control study Level: III-3 Quality: 14.5/26 High risk of bias	N=107 (50 in NAAT group and 57 in non-NAAT group) Median age NAAT: 46.5 years, non-NAAT: 47 years 20 (40%) females in NAAT group, 11 (19.3%) females in non-NAAT group 18 (36%) HIV infected in NAAT group (10 unknown), 19 (33.3%) HIV infected in non-NAAT group (10 unknown)	Inclusion: AFB-positive pulmonary TB suspects undergoing initial diagnostic evaluation between December 2000 and March 2006 Exclusion: Anti-TB therapy for > 6 days prior to sputum collection	Amplified MTD Direct Test, AFB microscopy and culture for MTB	AFB microscopy and culture	Unnecessary TB treatment time Concordance between MTB results and definitive diagnosis, compared with no MTB results

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Hanrahan et al. (2013) University of North Carolina Gillings School of Global Public Health, Chapel Hill, North Carolina, USA Conducted at: Primary care clinic in Johannesburg, South Africa	Prospective cohort study Level: III-3 Quality: 16.5/26 Some risk of bias	N=641 (50 NAAT-positive, 591 NAAT-negative) Median age: 35 years (IQR 29–44) 415 (65%) females 443 (69%) HIV infected 36 (6%) unknown	Inclusion: TB suspects presenting at the clinic, providing consent Exclusion: Not reported	Xpert MTB/RIF assay, sputum AFB FL microscopy and liquid culture for MTB	NA	Number of cases starting TB treatment Median time to TB treatment
Kwak et al. (2013) Division of Pulmonary and Critical Care Medicine, Department of Internal Medicine, Seoul National University College of Medicine, Seoul, Republic of Korea Conducted at: Seoul National University Hospital	Retrospective cohort Level: III-3 Quality: 16.5/26 Some risk of bias	N=681 patients requested for NAAT Median age: 61 years (IQR 47.5–73.0) 255 (37.4%) females 5 (0.7%) HIV infected	Inclusion: Patients in whom NAAT was requested due to suspicion of pulmonary TB between 1 January 2011 and 31 May 2013 Exclusion: Not reported	Xpert MTB/RIF assay, mycobacterial culture (liquid and/or solid) and AFB microscopy	NA	Time to report of results from laboratory Time to confirmation of results by physician Time to treatment
Lacroix et al. (2008) University of Sherbrooke, Sherbrooke, Quebec, Canada Conducted at: Public Health Department in Montegrie (Quebec)	Retrospective cohort Level: III-3 Quality: 12/26 High risk of bias	N=115/134 included (77 NAAT, 38 no NAAT) 43 (37.4%) females 7 (9.9%) HIV infected	Inclusion: Contagious (pulmonary, laryngeal, miliary) active TB cases declared to the Public Health Department between 1 January 1998 and 30 June 2007 Exclusion: Non-respiratory TB, clinical case not confirmed by culture or PCR, incomplete file, previous episode of TB, incidentally found cases	PCR (in-house, not specified)	No PCR (culture, AFB microscopy, chest X-ray)	Average delay in diagnosis

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Lippincot et al. (2014) Institute for Global Health and Infectious Diseases, University of North Carolina at Chapel Hill, USA Conducted at: University of North Carolina Hospital	Prospective cohort Level: III-3 Quality: 18/26 Some risk of bias	N=207/246 included Median age: 51 years (IQR 39–63), 74 (35.8%) females 49 (23.7%) HIV infected 31 (15%) unknown	Inclusion: Consecutive inpatient adults with presumptive TB, for whom at least one sputum specimen was submitted Exclusion: Patients with cystic fibrosis	Xpert MTB/RIF assay, AFB microscopy and culture	NA	Median laboratory processing time
Marks et al. (2013) US Centers for Disease Control and Prevention, Atlanta, Georgia, USA Conducted at: Metropolitan Atlanta, Georgia, four areas of Maryland and Massachusetts	Retrospective cohort Level: III-2 Quality: 16.5/26 Some risk of bias	N=2,140 (920 NAAT) 880 (41%) females 353 (25%) HIV infected	Inclusion: Suspected pulmonary TB in 2008–10 Exclusion: Patients lacking AFB microscopy/culture results	NAAT (MTD, Gen-Probe, San Diego, California), AFB microscopy and culture	(No NAAT) AFB microscopy and/or culture	Change in management after negative NAAT Change in management after positive NAAT Average outpatient days on TB medication (vs no NAAT) Differences in procedures Days to final TB determination
Omrani et al. (2014) Prince Sultan Military Medical City, Riyadh, Saudi Arabia	Retrospective cohort Level: III-3 Quality: 17/26 Some risk of bias	N=140 (76 NAAT, 64 no NAAT) Median age: 44.5 years (range 13–97) 61 (44%) females 0 HIV infected 44 (38.6%) pulmonary TB, 86 (61.4%) extrapulmonary TB	Inclusion: Patients who were commenced on anti-TB therapy for a diagnosis of active TB between 1 March 2011 and 28 February 2013 Exclusion: Not reported	Xpert MTB/RIF assay, with/without AFB microscopy and/or culture	Mycobacterial culture and/or AFB microscopy	Impact on time to start anti-TB treatment Rate of discontinuing treatment after negative NAAT
Sohn et al. (2014) McGill International TB Centre and McGill University, Montreal, Canada	Prospective cohort Level: III-3 Quality: 16.5/26 Some risk of bias	N=502 Median age: 44 years (IQR 31–61 years) 223 (44.4%) females 12 (2.4%) HIV infected	Inclusion: Patients aged > 17 years for evaluation of suspected active pulmonary TB Exclusion: Not reported	Xpert MTB/RIF assay	AFB microscopy and/or culture	Time to test result Time to treatment initiation Impact on treatment given

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Taegtmeyer et al. (2008) Tropical and Infectious Disease Unit, Royal Liverpool University Hospital, Liverpool, UK	Retrospective cohort study Level: III-3 Quality: 14.5/26 High risk of bias	N=87 patients were indicated for NAAT (AFB +ve) 51 received NAAT, 36 no NAAT	Inclusion: Patients with AFB-positive clinical samples submitted between January 2002 and December 2006 Exclusion: Not reported	NAAT using the INNO-LiPA Rif.TB assay (Immunogenetics, Zwijndrecht, Belgium)	AFB microscopy and/or mycobacterial culture	Time to identification of TB and rifampicin resistance Change in treatment
Theron et al. (2014) University of Cape Town, South Africa Conducted at: Five primary healthcare facilities in areas of southern Africa with a high HIV prevalence	Randomised controlled trial (multicentre) Level: II Quality: 23/26 Low risk of bias	N=1,502 Median age: 37 years (IQR 30–46) 643 (43%) females 895 (60%) HIV infected 758 assigned to AFB microscopy 744 assigned to Xpert MTB/RIF	Inclusion: > 17 years of age, one or more symptoms of pulmonary TB (according to WHO criteria), able to provide sputum specimens, no anti-TB treatment in the past 60 days Exclusion: Not reported	Xpert MTB/RIF assay on sputum specimen by nurse who received a 1-day training session	AFB microscopy on sputum specimen Positive if any smear revealed AFB over 100 fields (1000x for light microscopy and 400x for fluorescence microscopy)	Treatment initiation at baseline Treatment initiation as a result of clinical evidence % of TB patients not initiating treatment Time to treatment initiation
Van Rie et al. (2013a) Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, USA Conducted at: Witkoppen Health and Welfare Centre, Johannesburg, South Africa	Prospective cohort study Level: III-3 Quality: 13.5/26 High risk of bias	N=160/180 had valid results Median age: 36 years (IQR 30–44 years) 113 (57%) females 144 (72%) HIV infected	Inclusion: TB suspects who were AFB-negative and returned for their result Exclusion: Not reported	Xpert MTB/RIF assay Patients also underwent fluorescent AFB microscopy and liquid culture	-	Time to treatment initiation
Van Rie et al. (2013b) Department of Epidemiology, University of North Carolina, Chapel Hill, North Carolina, USA	Prospective cohort study Level: III-3 Quality: 19.5/26 Some risk of bias	N=344 patients, with 162 positive Xpert FNAs Age: 53% were < 36 years 164 (49%) females 100% were HIV infected	Inclusion HIV-infected, clinically suspected of lymph node TB, aged > 17 years, not receiving treatment for active or latent TB	Xpert MTB/RIF Patients also underwent AFB microscopy (ZN and FL staining) and	NA	CIM: median time of FNA collection and diagnosis Time to treatment initiation

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Conducted at: Helen Joseph Hospital, Johannesburg, South Africa				mycobacterial culture (MGIT medium)		
Yoon et al. (2012) Division of Pulmonary and Critical Care Medicine, San Francisco General Hospital, University of San Francisco, San Francisco, California, USA Conducted at: Mulago Hospital, Kampala, Uganda	Historical cohort study Level: III-3 Quality: 18.5/26 Some risk of bias	N=477/525 patients Median age: 33 years (IQR 27–40) 229 (48%) female 362 (76%) HIV infected	Inclusion: Consecutive adults > 17 years of age admitted to hospital with cough for >2 weeks but < 6 months duration, and provided consent Exclusion: Receiving TB treatment at the time of enrolment, no available culture results, no NAAT on implementation phase, death within 3 days of hospital admission	Xpert MTB/RIF assay, sputum AFB microscopy and mycobacterial culture	Same tests, but in comparator group Xpert results were not reported to clinicians or used for patient management	Time to TB detection Time to TB treatment

CIM = change in management; FL = fluorescent; FNA = fine-needle aspirate; HIV = human immunodeficiency virus; IQR = interquartile range; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; NTM = non-tuberculous mycobacteria; RIF = rifampicin; TB = tuberculosis; WHO = World Health Organization; ZN = Ziehl-Neelsen

Table 98 Study profiles of included studies on the effectiveness of change in management due to early treatment of TB in those with low pre-test probability of having active TB

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Ponticiello et al. (2001) Monaldi Hospital, Naples, Italy TB referral center for Campania	Prospective cohort study Level: III-2 Quality: 16/26 High risk of bias	N=90 cases of TB 100% Caucasian 48 (53%) had cavity type lesion on chest X-ray Average delay in diagnosis 2.25 ± 1.0 months 3 (2.7%) TST-negative Contacts: N=277 (out of 346 identified contacts) 44 did not comply with protocol 25 refused consent	Inclusion All patients with newly diagnosed pulmonary TB during the period January 1997 – December 1998 and their contacts (those sharing the same indoor environment for prolonged periods) AFB in sputum or bronchial smear and positive culture for MTB Exclusion Patients with HIV and their contacts No written informed consent Failure to comply with study protocol	Delay to diagnosis ≤ 1 month Delay defined as period from onset of any TB symptoms to diagnosis	Delay in diagnosis: 1.5 months 2.0 months (also examines up to 5 months)	Proportion of contacts TST-positive Proportion of contacts TST-negative Odds TST+/TST– OR (TST+/TST–) Various lengths of delay compared with reference ≤ 1 month delay Analysis of clinical risk factors ORs and their 95%CIs were calculated by means of univariate and multivariate logistic regression
Golub et al. (2006) Maryland, USA	Prospective cohort study Level: III-2 Quality: 16/26 High risk of bias	N=124 total patients with pulmonary TB included 34 excluded due to no contacts identified/tested N=54 (44%) born in USA US patients: 65% male 72% black 59% < 50 years of age 57% AFB sputum-positive 19% chest X-ray with cavitation 385 contacts, of whom 310 (81%) skin tested	Inclusion All verified pulmonary TB patients who reported to the Maryland Department of Health and Mental Hygiene between 1 June 2000 and 30 November 2001 and their close contacts Close contacts included those living in the same household, working in a closed environment with patient, and reported close friends and relatives Exclusion No contacts identified or no contacts tested	Treatment delay either: ≤ 60 days or ≤ 90 days Delay treated as a dichotomous variable, analysed for cut-offs of 60 and 90 days Total treatment delay defined as interval from first TB symptoms to initiation of treatment for TB	Treatment delay > 60 days Treatment delay > 90 days	Number of contacts infected TST-positive (tested at baseline and 10–12 weeks later)

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Van der Oest, Kelly & Hood (2004) Waikato Health District, New Zealand	Retrospective cohort study Level: III-2 Quality: 11/26 High risk of bias	N=244 (189 new cases, 37 relapse cases, 18 unclassified): 52% male 110 (45%) Maori 46 (19%) non-Indigenous New Zealanders 81 (33%) born overseas, 40 of whom were refugees number with length of diagnostic delay reported 152 (62% of cases) outcome of treatment reported for 214 (88%) of cases	Inclusion All notified cases of TB who were residing in the Waikato Health District at the time of notification from 1 January 1992 to 31 December 2001	No diagnostic delay Delay defined as time between development of symptoms and notification/diagnosis of the case	Increasing diagnostic delay	Favourable treatment outcome as defined by WHO (i.e. cure or treatment completed) Statistical analysis Logistic regression was used for multivariate and univariate comparisons Chi-square test

AFB = acid-fast bacilli; HIV = human immunodeficiency virus; OR = odds ratio; TB = tuberculosis; TST = tuberculin skin test; WHO = World Health Organization

Table 99 Study profiles of included studies on the effectiveness of change in management due to rifampicin-resistance mutations being identified

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Drobniewski et al. (2002) UK	Cohort study Level: III-2 Quality: 19/26 Some risk of bias	N=90 MDR-TB patients 36 born in UK, 7 in Pakistan, 5 in India, 4 in Bangladesh, 20 in Africa, 4 in Europe, 1 each from USA, Australia, Philippines, Japan, Trinidad, Jamaica All cases were resistant to at least isoniazid and RIF, and 29 and 33 cases were resistant to pyrazinamide and ethambutol, respectively	Inclusion All mycobacterial cultures identified by the Public Health Laboratory Service, Mycobacterium Reference Unit, Scottish Mycobacteria Reference Laboratory, and PHLS Regional Centres for Mycobacteria	Treatment with at least three drugs to which the bacterium was susceptible	Treatment with fewer drugs to which the bacterium was susceptible	Median survival period Chance of death

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Outcomes
Lam et al. (2014) Thailand	Retrospective chart review Level: III-2 Quality: 18.5/26 Some risk of bias	N=190 RIF-resistant or MDR-TB patients	Inclusion Patients with DST results demonstrating infection with RIF-resistant or MDR-TB who were registered for TB treatment during October 2004 – September 2008 at health facilities within the Thailand TBAactive Surveillance Network Exclusion Patients from health facilities operated by private practitioners, non-governmental organisations, or facilities serving solely as referral centres, patients with incomplete laboratory data and patients with NTM infection or a change in diagnosis	Treatment other than Category II	Category II treatment (streptomycin, isoniazid, ethambutol, RIF and pyrazinamide)	Odds for poor outcome (treatment fail, death)
Meyssonnier et al. (2014) France	Retrospective cohort study Level: III-2 Quality: 18/26 Some risk of bias	N=39 RIF-mono-resistant TB patients, data about treatment and outcome were available for 30 patients 19 males (49%), median age 43 years (IQR 29–58) Foreign born 18 (46%)	Inclusion All patients diagnosed with RIF-mono-resistant TB reported to the national network in France between 2005 and 2010	Treatment with antibiotics other than RIF	Treatment with RIF-containing antibiotic regimen	Health outcomes (recovery, lost to follow-up, death, relapse)

IQR = interquartile range; MDR = multidrug-resistant; NTM = non-tuberculous mycobacteria; RIF = rifampicin; TB = tuberculosis; DST = drug susceptibility testing

Table 100 Study profiles of SRs assessing the safety and adverse effects of active TB therapies

Author Country	Study design Quality appraisal	Research question Included studies (N)	Population	Intervention	Comparator	Outcomes
Forget and Menzies (2006) Canada	Systematic review (literature search of Medline, relevant articles from authors' files and pearled references from cited articles) Quality appraisal: poor High risk of bias	Research question: NS Included studies: NS	Active TB patients Latent TB patients TB patients on multidrug regimens	Isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin Multidrug regimens	Various	Discontinuation rate AE rate Mortality rate Risk factors for development of AEs
Frydenberg & Graham (2009) Australia	Systematic review (literature search of PubMed, EMBASE and Cochrane Library Reference, hand-search of reference lists) Quality appraisal: medium Some risk of bias	Research question: To review the frequency and manifestations of toxicities in children to current first-line anti-TB therapy Included studies: NS	Children (0–18 years of age) undergoing first-line therapy for TB, or therapy for latent TB	Anti-TB agents, isoniazid, rifampicin, pyrazinamide, ethambutol, streptomycin Combination therapies	NS	Adverse reaction incidence AE rate
van der Werf et al. (2012) The Netherlands	Systematic review and meta-analysis (methodology according to the Cochrane Handbook for Systematic Reviews and PRISMA) Quality appraisal: good Low risk of bias	Research question: To assess the risk of development of MDR-TB after the use of inappropriate TB regimens Included studies: 4 (2 studies included in meta-analysis)	Non-MDR patients receiving treatment for TB and who underwent drug-resistance measurement and genotype of the isolated MTB bacilli before treatment started, and drug resistance and genotype in failure and/or recurrent TB cases	Inappropriate treatment regimens for diagnosed non-MDR-TB	Appropriate treatment regimens for non-MDR-TB	MDR-TB

AE = adverse events; MDR = multidrug-resistant; NS = not stated; TB = tuberculosis

Table 101 Study profiles of included studies on the effectiveness of NAAT in diagnosing NTM infections

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Reference standard
Abdalla et al. (2009) Brazil	Level II: A comparison against independent, blinded reference standard among consecutive patients Quality: High risk of bias Patient selection ☹ Index test ☹ Comparator ? Reference std ☺ Flow and timing ☹ Applicability: C1, P2	N=34 FFPE skin biopsy specimens from 32 consecutive patients suspected of cutaneous TB or atypical mycobacterial infection Aged 14–79 years 14/32 male	Inclusion All consecutive patients suspected of cutaneous TB or atypical mycobacterial infection who attended the Dermatologic Clinic of the University of São Paulo Medical School Hospital during the period January 2001 – January 2004	PCR using an assay based on oligonucleotide primers specific for the 65-kDa antigen gene of mycobacteria on DNA extracted from FFPE tissue to detect NTM	Results of AFB microscopy were obtained from patient records	Results of L-J culture were obtained from patient records Also used a clinical reference standard defined as successful treatment for NTM
Bogner et al. (1997) Germany Multicentre study of AIDS treatment centres	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: Low risk of bias Patient selection ☺ Index test ☺ Reference std ? Comparator ☹ Flow and timing ☺ Applicability: C1, P1	N=540 blood specimens from 107 AIDS patient suspected of having MAC infection, recruited between May 1994 and May 1995, followed up for average of 17.2 ± 11 weeks Mean age 40.3 ± 9.2 years Majority male	Inclusion HIV infection, age > 18 years, either symptoms suggestive of MAC disease without other explanation, or MAC positivity in one specimen but no symptoms Exclusion Patients with MTB or MAC disease in past, use of anti-TB drugs in previous 8 weeks, life expectancy of < 4 weeks and low Karnofsky index of < 50%	PCR (using not-commercially available PCR test kids by Roche) of blood samples to detect *Mycobacterium avium*	Not done	Culture of specimens grown in BACTEC 12B vials and on L-J media
Choi et al. (2012) Korea	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☹ Index test ☺ Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P2	N=531 respiratory specimens from 230 patients with suspected mycobacterial infection 482 sputum 49 BAL	Inclusion People with suspected MTB as well as other mycobacteria in July and August 2011	Respiratory specimens tested with qPCT-based assay (PNAqPCR TB/NTM kit) targeting the 16S-23S rRNA internal transcribed spacer region to detect NTM	AFB microscopy with AUR stain	Respiratory specimens cultured in BACTEC MGIT 960 for 6 weeks at 36 ºC

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Reference standard
Frevel et al. (1999) Germany	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: High risk of bias Patient selection ☹ Index test ☹ Comparator ☹ Reference std ☺ Flow and timing ☹ Applicability: C1, P1	N=69 FFPE pulmonary and extrapulmonary samples from patients who were suspected of mycobacterial infections	Inclusion 229 FFPE samples from 141 patients who, either for clinical (51%) or histological (49%) reasons, were suspected of MTB or NTM infections initially included in study	Pulmonary and extrapulmonary specimens tested with PCR, targeting gene for 65-kDa heat shock protein to detect NTM	AFB microscopy using ZN staining was done routinely but results not reported	The microbiological results were obtained from patient records
Gamboa et al. (1997) Spain	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☹ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=136 blood specimens from AIDS patients suspected of having disseminated mycobacterial infection Median age 31 years (range 2–55) 80% male	Inclusion Blood specimens and BM aspirates from AIDS patients who were suspected of having disseminated mycobacterial infections and were not receiving anti-TB therapy, and attended April–December 1996	Blood and BM specimens tested using the Roche Amplicor MAI PCR-based test to detect *Mycobacterium avium* and *M. intracellulare*	AFB microscopy using AUR and positive slides confirmed with ZN staining	BACTEC 13A cultures were incubated at 37 °C for 8 weeks, and examined for growth with the BACTEC 460 radiometer twice weekly for the first 2 weeks and once weekly thereafter
Gazzola et al. (2008) Italy	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☹ Index test ? Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P1	N=71 blood samples from 65 AIDS patients suspected of having disseminated mycobacterial infection N=46 BM specimens from 41 AIDS patients	Inclusion Between March 1999 and April 2004 all episodes of suspected disseminated mycobacterial infections in AIDS patients	Blood and BM specimens tested with PCR to detect *Mycobacterium avium* (no details provided)	AFB microscopy using ZN staining for BM specimens only	Culture of blood and BM specimens (radiometric BACTEC AFB system) CRS was clinical diagnosis based on suggestive signs and symptoms plus histopathologic results and response to anti-MAC therapy
Kox et al. (1997) The Netherlands	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias	N=259 samples from 177 patients: 31 sputum specimens 87 biopsy specimens 37 lymph node biopsies	Inclusion Patients for whom difficulties with diagnosis were experienced or could be anticipated (e.g. with granulomatous disease,	The PCR assays were performed at the Royal Tropical Institute, targeting the 16S DNA sequence to detect	AFB microscopy done according to standard	Culture done according to standard methods at laboratories of hospitals to which

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Reference standard
	Patient selection ☹ Index test ☺ Comparator ? Reference std ? Flow and timing ☺ Applicability: C1, P1	7 faeces specimens 6 urine specimens 10 blood samples 36 CSF specimens 6 ascitic fluid 15 pleural fluid 3 pericardial fluid 20 BAL 1 gastric lavage	suspected extrapulmonary TB), immunocompromised patients (i.e. HIV-positive or with AIDS), immigrants and refugees from countries with high incidence of TB, and patients in whom mycobacterial infection other than by MTB was suspected	NTM, and results were reported to the clinicians within 3 days	methods at laboratories of hospitals to which patients were referred	patients were referred CRS defined as clinical assessment after resolution of discrepancies
Mahaisavariya et al. (2005) Thailand	Level III-1: A comparison against independent, blinded reference standard among non-consecutive patients Quality: High risk of bias Patient selection ☹ Index test ☹ Comparator ? Reference std ☺ Flow and timing ☹ Applicability: C1, P2	N=131 FFPE tissues from 111 patients Only 120 specimens were cultured at time of processing Mean age 34.6 years (range 2–73) 58 males	Inclusion Patients with suspected mycobacterial infections (e.g. asymptomatic and slowly progressive skin lesions or cervical lymphadenitis with draining sinus) who attended the Granuloma Clinic, Siriraj Hospital, Mahidol University, Bangkok, between 1994 and 2000 Exclusion Cases with leprosy and deep fungal infection	DNA extracted from FFPE specimens One-tube nested and multiplex PCR using 16S rRNA sequence as the target to detect NTM	Histopathologic sections were reviewed blindly for AFB detection by two independent observers	Culture results were retrieved from the patient records
Matsumoto et al. (1998) Japan	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=141 bronchial wash specimens from 127 patients All were HIV–	Inclusion Retrospective analysis of bronchial washing specimens collected from patients suspected of mycobacteriosis, peripheral lung cancer or other miscellaneous pulmonary diseases from August 1995 to March 1997 Exclusion Patients with a final diagnosis of TB	PCR using Amplicor PCR assay to detect *Mycobacterium avium*	AFB microscopy using ZN staining	Culture on Ogawa egg medium for up to 8 weeks

Study setting	Study design Quality appraisal	Study population	Selection criteria	Intervention	Comparator	Reference standard
Ninet et al. (1997) Switzerland	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: High risk of bias Patient selection ☹ Index test ☺ Comparator ☹ Reference std ? Flow and timing ☹ Applicability: C1, P1	N=201 blood samples from HIV-infected patients	Inclusion Retrospective study conducted in the Division of Infectious Disease, Hospital Cantonal Universitaire, Geneva, using blood samples from HIV-infected patients collected over a 2-year period	DNA isolated from blood was stored frozen prior to use in PCR with Amplicor MAI (Roche) Tested for *Mycobacterium avium, M. intracellulare* and MTB	Not done	Culture of whole blood in BACTEC 13A medium
Phillips et al. (2005) Ghana	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Low risk of bias Patient selection ☺ Index test ☺ Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P2	N=70 biopsy specimens from 70 patients	Inclusion Punch biopsy specimens were obtained from subjects with a strong clinical suspicion of *Mycobacterium ulcerans* disease (Buruli ulcer) prior to treatment of the lesion by excision, who presented at St Martin's Hospital, Agroyesum, Nkawie Hospital and Tepa Hospital between September 2003 and June 2004	PCR to detect *M. ulcerans* targeting IS*2404*	AFB microscopy using ZN staining	L-J slopes were incubated at 31 °C, and the cultures were examined weekly until visible growth occurred CRS was defined as histological diagnosis, or a positive culture for *M. Ulcerans*
Tran et al. (2014) USA	Level III-2: A comparison with reference standard (not blinded or blinding not known) Quality: Some risk of bias Patient selection ☹ Index test ? Comparator ☺ Reference std ? Flow and timing ☺ Applicability: C1, P1	N=464 respiratory specimens (sputum and bronchial washes)	Inclusion Specimens received in the Mycobacteriology Laboratory at the Wadsworth Center, New York State, between 1 May 2012 and 1 February 2013 including MTB culture-positive specimens	MTBC-MAC multiplex qPCR using 1 forward primer, 5 reverse primers and 2 probes designed to detect the 16S-23S rRNA internal transcribed spacer region of all MAC strains	AFB microscopy using ZN staining	Specimens were cultured using the BACTEC MGIT 960 system

AIDS = acquired immunodeficiency syndrome; AUR = auramine-based fluorochrome; BAL = bronchoalveolar lavage; BM = bone marrow; CRS = clinical reference standard; CSF = cerebrospinal fluid; FFPE = formalin fixed, paraffin embedded; L-J = Lowenstein-Jensen; MAC = *Mycobacterium avium* complex; MGIT= Mycobacterium Growth Indicator Tubes; MTB = *Mycobacterium tuberculosis*; NALC-NaOH = N-acetyl-L-cysteine-sodium hydroxide; qPCR = real-time PCR; PCR = polymerase chain reaction; ZN = Ziehl-Neelsen

Appendix G Excluded studies

Studies published from 2005 onwards that met the PICO criteria to assess the diagnostic accuracy of AFB microscopy and NAAT compared with culture, but were excluded for the reasons listed below.

No 2x2 data

Abdelwahab, AE 2009, 'Immunological and molecular diagnosis of *Mycobacterium tuberculosis* between two environmentally different regions', *Current Research in Tuberculosis*, vol. 1, no. 1, pp. 7–14.

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Baxi, A, Neema, H, Kaushal, M, Sahu, P & Baxi, D 2011, 'Genital tuberculosis in infertile women: assessment of endometrial TB PCR results with laparoscopic and hysteroscopic features', *Journal of Obstetrics and Gynecology of India*, vol. 61, no. 3, pp. 301–306.

Bhanothu, V, Theophilus, JP, Reddy, PK & Rozati, R 2014, 'Occurrence of female genital tuberculosis among infertile women: a study from a tertiary maternal health care research centre in South India', *European Journal of Clinical Microbiology & Infectious Diseases*.

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Appendix H Economic literature search

Literature search for previously published economic evaluations of NAAT

(tuberculosis OR MTB OR MTB/RIF OR "tubercle bacillus" OR Tuberculosis [MeSH] OR "M. bovis" OR "M. africanum" OR "M. microti" OR "M. canetti") AND (Amplicor OR Amplified OR "Direct Test" OR "Direct Detection" OR TaqMan OR Xpert OR "nucleic acid amplification" OR NAAT OR "Nucleic Acid Amplification Techniques" [MeSH]) AND (economic evaluation OR cost effectiveness OR cost utility OR decision model)

Database	Last updated
Centre for Reviews and Dissemination database – including Database of Abstracts of Reviews of Effects, the Health Technology Assessment Database, the NHS Economic Evaluation Database	19 May 2014
PubMed	19 May 2014

Literature search for utility weights in TB

Tuberculosis AND (quality of life OR utility OR EQ-5D OR euroqol OR time trade off OR standard gamble OR visual analogue scale OR VAS OR SF-36 OR SF-6D OR SF 6D OR SF 36 OR EQ 5D)

Database	Last updated
PubMed	15 August 2014

Appendix I Additional information relating to the economic evaluation

Amikacin administration costs

Table 102 Amikacin administration costs

	False-positive MDR-TB	True MDR-TB
Average administrations per week	5	4
Weeks treated	8.7	26.1
Total amikacin administrations	43.5	104.4
In-patient administrations	9.3	14.9
Outpatient administrations	34.2	89.5
Cost of outpatient administration	$234	$234
Total administration costs	$8,002	$20,943

MDR-TB = multidrug-resistant tuberculosis

Utility values

Table 103 Utility values identified in studies that elicit utilities in a TB population

Study	Utility weight		Comments
Winetsky et al. (2012)	No TB / recovered Active TB ± MDR, undiagnosed Active TB ± MDR, AFB– treated Active TB ± MDR, AFB+ treated	1.00 0.73 0.68 0.60	Former Soviet Union prison inmates—unclear how preferences were elicited
Kittikraisak et al. (2012)	TB (treated) MDR-TB (treated) Cured TB ± MDR HIV (no TB) HIV with TB (treated) Cured TB ± MDR with HIV	0.65 0.49 0.89 0.75 0.62 0.88	Thai patients diagnosed with TB (including those on treatment and those who had completed treatment) and/or HIV EQ-5D weights adjusted for age and monthly household income (also presents unadjusted weights with standard error)
Awaisu et al. (2012)	At baseline At 3 months treatment At 6 months treatment	0.70 0.87 0.91	Malaysian smokers newly diagnosed with TB EQ-5D weights for control group (treatment administered by DOTS)
Kruijshaar et al. (2010) (reported in Jit et al. (2011))	Untreated TB After 2 months treatment	0.68 0.81	UK patients diagnosed with active TB were administered generic health-related quality-of-life questionnaires (EQ-5D and SF-36) at diagnosis and 2 months into therapy. The median time between questionnaires was 73 days (IQR: 54-104) Overall scores are not reported in Krujisharr et al. (2010), but are reported in Jit et al. (2011)
Babikako et al. (2010)	At baseline At 2 months treatment At 8 months treatment	0.607 0.671 0.785	Ugandan patients with known HIV status and TB enrolled based on duration of TB therapy: started at 2 months and completed at 8 months

Study	Utility weight		Comments
Guo et al. (2008)	Active TB (treated) 0.72)	0.68 (0.65–	Canadian patients with recent diagnosis of active TB (within 2 months) Measure using the SF-6D (also HUI2, HUI3 and VAS) Also reports by self-reported symptom control
	By severity of TB symptoms:		
	Very mild	0.84 (0.11)	
	Mild	0.68 (0.05)	
	Moderate	0.64 (0.10)	
	Severe	0.59 (0.15)	
	Very severe	0.54 (0.08)	

AFB = acid-fast bacilli; DOTS = directly observed treatment, short-course; HIV = human immunodeficiency virus; IQR = inter-quartile range; MDR = multidrug-resistant; MDR-TB = multidrug-resistant tuberculosis; TB = tuberculosis; VAS = visual analogue scale

Results of the economic evaluation

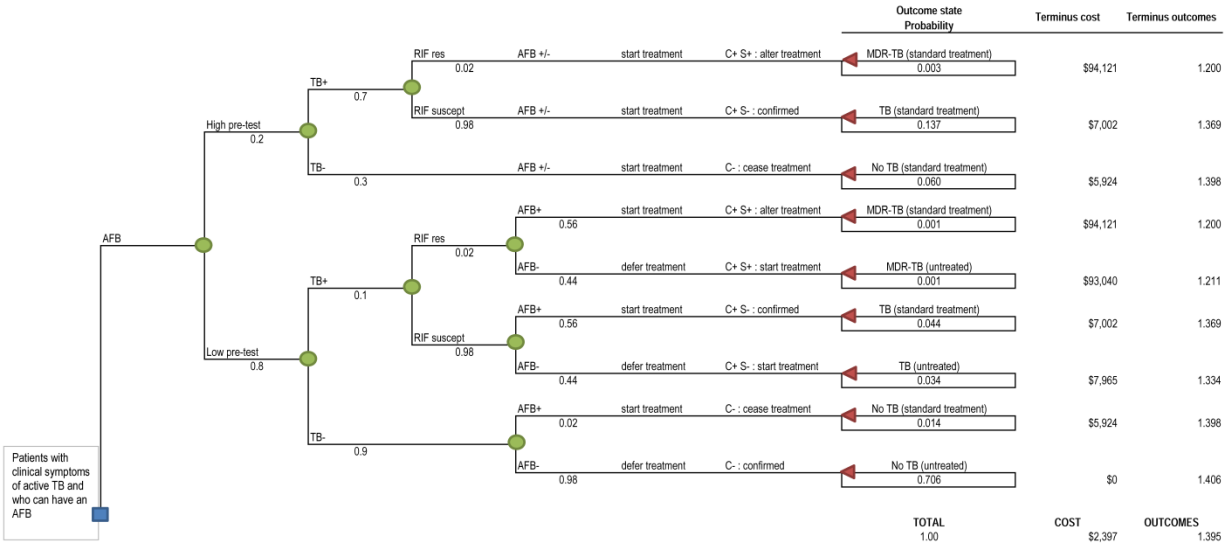

Figure 55 Results of the economic evaluation, AFB model arm

AFB = acid-fast bacilli test; C = culture; MDR = multidrug-resistant; R = resistance; RIF res = rifampicin-resistant; RIF suscept = rifampicin-susceptible; S = susceptibility; TB = tuberculosis

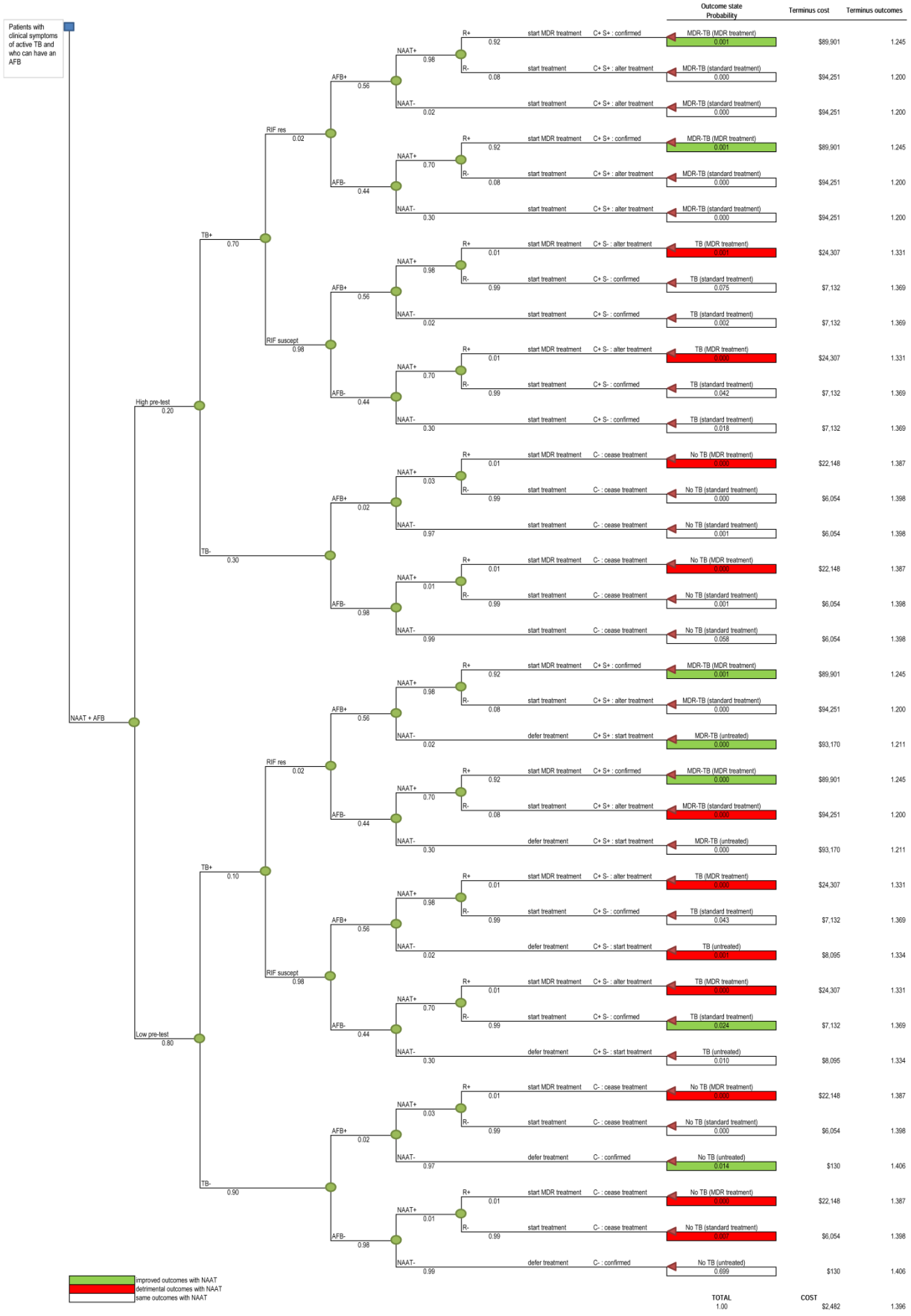
Outcome state
Probability
Terminus cost
Terminus outcomes
Patients with clinical symptoms of active TB and who can have an AFB
NAAT + AFB
High pre-test 0.20
Low pre-test 0.80
TB+ 0.70
TB- 0.30
TB+ 0.10
TB- 0.90
RIF res 0.02
RIF suscept 0.98
AFB+ 0.56
AFB- 0.44
NAAT+ 0.98
NAAT- 0.02
NAAT+ 0.70
NAAT- 0.30
NAAT+ 0.03
NAAT- 0.97
NAAT+ 0.01
NAAT- 0.99
R+ 0.92
R- 0.08
R+ 0.01
R- 0.99
start MDR treatment
start treatment
defer treatment
C+ S+ : confirmed
C+ S+ : alter treatment
C+ S+ : start treatment
C+ S- : alter treatment
C+ S- : confirmed
C- : cease treatment
C- : confirmed
MDR-TB (MDR treatment) 0.001 $89,901 1.245
MDR-TB (standard treatment) 0.000 $94,251 1.200
MDR-TB (standard treatment) 0.000 $94,251 1.200
MDR-TB (MDR treatment) 0.001 $89,901 1.245
MDR-TB (standard treatment) 0.000 $94,251 1.200
MDR-TB (standard treatment) 0.000 $94,251 1.200
TB (MDR treatment) 0.001 $24,307 1.331
TB (standard treatment) 0.075 $7,132 1.369
TB (standard treatment) 0.002 $7,132 1.369
TB (MDR treatment) 0.000 $24,307 1.331
TB (standard treatment) 0.042 $7,132 1.369
TB (standard treatment) 0.018 $7,132 1.369
No TB (MDR treatment) 0.000 $22,148 1.387
No TB (standard treatment) 0.000 $6,054 1.398
No TB (standard treatment) 0.001 $6,054 1.398
No TB (MDR treatment) 0.000 $22,148 1.387
No TB (standard treatment) 0.001 $6,054 1.398
No TB (standard treatment) 0.058 $6,054 1.398
MDR-TB (MDR treatment) 0.001 $89,901 1.245
MDR-TB (standard treatment) 0.000 $94,251 1.200
MDR-TB (untreated) 0.000 $93,170 1.211
MDR-TB (MDR treatment) 0.000 $89,901 1.245
MDR-TB (standard treatment) 0.000 $94,251 1.200
MDR-TB (untreated) 0.000 $93,170 1.211
TB (MDR treatment) 0.000 $24,307 1.331
TB (standard treatment) 0.043 $7,132 1.369
TB (untreated) 0.001 $8,095 1.334
TB (MDR treatment) 0.000 $24,307 1.331
TB (standard treatment) 0.024 $7,132 1.369
TB (untreated) 0.010 $8,095 1.334
No TB (MDR treatment) 0.000 $22,148 1.387
No TB (standard treatment) 0.000 $6,054 1.398
No TB (untreated) 0.014 $130 1.406
No TB (MDR treatment) 0.000 $22,148 1.387
No TB (standard treatment) 0.007 $6,054 1.398
No TB (untreated) 0.699 $130 1.406
improved outcomes with NAAT
detrimental outcomes with NAAT
same outcomes with NAAT
TOTAL
1.00
COST
$2,482
1.396

Figure 56 Results of the economic evaluation, AFB plus NAAT model arm

AFB = acid-fast bacilli test; C = culture; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; R = resistance; RIF res = rifampicin-resistant; RIF suscept = rifampicin-susceptible; S = susceptibility; TB = tuberculosis

Additional scenarios

TB low-suspicion scenario

Table 104 Outcome state probabilities, TB low scenario

True status	Treated status	AFB	AFB + NAAT	Difference
No TB	Untreated	76.4%	77.2%	0.75%
	Standard treatment	1.6%	0.8%	–0.76%
	MDR treatment	0.0%	0.0%	0.01%
TB	Untreated	9.5%	3.1%	–6.40%
	Standard treatment	12.1%	18.3%	6.21%
	MDR treatment	0.0%	0.2%	0.18%
MDR-TB	Untreated	0.2%	0.1%	–0.13%
	Standard treatment	0.2%	0.0%	–0.22%
	MDR treatment	0.0%	0.3%	0.35%
TOTAL		100%	100%	0%

Note: The probabilities associated with the correct treatment are highlighted.

AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 105 Breakdown of incremental costs, TB low scenario

Cost	AFB	AFB + NAAT	Increment
Treatment	$556.06	$580.41	$24.36
Treatment of AEs	$3.02	$2.97	–$0.05
Management	$174.78	$172.86	–$1.92
Hospitalisation	$1,212.03	$1,176.62	–$35.40
TB transmissions	$159.54	$85.96	–$73.58
NAAT cost	$0.00	$130.00	$130.00
TOTAL	$2,105.43	$2,148.83	$43.39

AEs = adverse events; AFB = acid fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 106 Weighted utility by outcome state, TB low scenario

True status	Treated status	AFB	AFB + NAAT	Increment
No TB	Untreated	1.075	1.085	0.011
	Standard treatment	0.022	0.011	–0.011
	MDR treatment	0.000	0.000	0.000
TB	Untreated	0.127	0.041	–0.085
	Standard treatment	0.165	0.250	0.085
	MDR treatment	0.000	0.002	0.002

True status	Treated status	AFB	AFB + NAAT	Increment
MDR-TB	Untreated	0.002	0.001	–0.002
	Standard treatment	0.003	0.000	–0.003
	MDR treatment	0.000	0.004	0.004
TOTAL		1.394	1.396	0.002

Note: The outcomes associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Perfect clinical judgement scenario

Table 107 Outcome state probabilities, perfect clinical judgement scenario

True status	Treated status	AFB	AFB + NAAT	Difference
No TB	Untreated	76.4%	77.2%	0.75%
	Standard treatment	1.6%	0.8%	–0.76%
	MDR treatment	0.0%	0.0%	0.01%
TB	Untreated	0.0%	0.0%	0.00%
	Standard treatment	21.6%	21.4%	–0.18%
	MDR treatment	0.0%	0.2%	0.18%
MDR-TB	Untreated	0.0%	0.0%	0.00%
	Standard treatment	0.4%	0.1%	–0.35%
	MDR treatment	0.0%	0.3%	0.35%
TOTAL		100%	100%	0%

Note: The probabilities associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 108 Breakdown of incremental costs, perfect clinical judgement scenario

Cost	AFB	AFB + NAAT	Increment
Treatment	$557.70	$580.95	$23.25
Treatment of AEs	$3.05	$2.98	–$0.07
Management	$175.21	$173.00	–$2.21
Hospitalisation	$1,212.03	$1,176.62	–$35.40
TB transmissions	$68.22	$56.24	–$11.98
NAAT cost	$0.00	$130.00	$130.00
TOTAL	$2,016.20	$2,119.79	$103.59

AEs = adverse events; AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 109 Weighted utility by outcome state, perfect clinical judgement scenario

True status	Treated status	AFB	AFB + NAAT	Increment
No TB	Untreated	1.075	1.085	0.011
	Standard treatment	0.022	0.011	–0.011
	MDR treatment	0.000	0.000	0.000

True status	Treated status	AFB	AFB + NAAT	Increment
TB	Untreated	0.000	0.000	0.000
	Standard treatment	0.295	0.293	–0.003
	MDR treatment	0.000	0.002	0.002
MDR-TB	Untreated	0.000	0.000	0.000
	Standard treatment	0.005	0.001	–0.004
	MDR treatment	0.000	0.004	0.004
TOTAL		1.397	1.397	0.0001

Note: the outcomes associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

TB high-suspicion scenario

Table 110 Outcome state probabilities, TB high scenario

True status	Treated status	AFB	AFB + NAAT	Difference
No TB	Untreated	0.0%	0.0%	0.00%
	Standard treatment	78.0%	78.0%	–0.01%
	MDR treatment	0.0%	0.0%	0.01%
TB	Untreated	0.0%	0.0%	0.00%
	Standard treatment	21.6%	21.4%	–0.18%
	MDR treatment	0.0%	0.2%	0.18%
MDR-TB	Untreated	0.0%	0.0%	0.00%
	Standard treatment	0.4%	0.1%	–0.35%
	MDR treatment	0.0%	0.3%	0.35%
TOTAL		100%	100%	0.00%

Note: The probabilities associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 111 Breakdown of incremental costs, TB high scenario

Cost	AFB	AFB + NAAT	Increment
Treatment	$1,206.95	$1,236.56	$29.61
Treatment of AEs	$12.44	$12.46	$0.02
Management	$404.91	$404.95	$0.04
Hospitalisation	$4,826.22	$4,826.22	$0.00
TB transmissions	$93.90	$82.17	–$11.73
NAAT cost	$0.00	$130.00	$130.00
TOTAL	$6,544.41	$6,692.36	$147.95

AEs = adverse events; AFB = acid-fast bacilli; NAAT = nucleic acid amplification test; TB = tuberculosis

Table 112 Weighted utility by outcome state, TB high scenario

True status	Treated status	AFB	AFB + NAAT	Increment
No TB	Untreated	0.000	0.000	0.000
	Standard treatment	1.091	1.091	0.000
	MDR treatment	0.000	0.000	0.000
TB	Untreated	0.000	0.000	0.000
	Standard treatment	0.295	0.293	–0.003
	MDR treatment	0.000	0.002	0.002
MDR-TB	Untreated	0.000	0.000	0.000
	Standard treatment	0.005	0.001	–0.004
	MDR treatment	0.000	0.004	0.004
TOTAL		1.391	1.391	0.0001

Note: The outcomes associated with the correct treatment are highlighted.
AFB = acid-fast bacilli; MDR = multidrug-resistant; NAAT = nucleic acid amplification test; TB = tuberculosis

Incorporating costs in a stepped manner

Table 113 Stepped economic evaluation, TB low and TB high scenarios

Utilities considered	Costs included (NAAT cost applied in AFB + NAAT arm)	ICER
TB low scenario		
Index patient utility	Treatment only	$75,861
	Treatment and AEs	$75,835
	Treatment, AEs and management	$74,891
	Treatment, AEs, management and hospitalisation	$57,491
	Treatment, AEs, management, hospitalisation and transmission	$21,327
Index and secondary case utility	Treatment, AEs, management, hospitalisation and transmission	$18,533
Perfect clinical judgment scenario		
Index patient utility	Treatment only	$1,174,732
	Treatment and AEs	$1,174,199
	Treatment, AEs and management	$1,157,291
	Treatment, AEs, management and hospitalisation	$885,893
	Treatment, AEs, management, hospitalisation and transmission	$794,067
Index and secondary case utility	Treatment, AEs, management, hospitalisation and transmission	$724,423
TB high scenario		
Index patient utility	Treatment only	$2,163,187
	Treatment and AEs	$2,163,492
	Treatment, AEs and management	$2,164,093
	Treatment, AEs, management and hospitalisation	$2,164,093
	Treatment, AEs, management, hospitalisation and transmission	$2,005,150
Index and secondary case utility	Treatment, AEs, management, hospitalisation and transmission	$1,713,838

AEs = adverse events; ICER = incremental cost-effectiveness ratio

Appendix J Alternative NAAT fees

Table 114 The effect on the ICER of alternative NAAT item fees

NAAT fee	ICER
$28.65	Dominant
$40.00	Dominant
$70.00	$26,768
$82.00	$39,560
$100.50	$59,281
$130.00	$90,728
$200.00	$165,348

Note: Figures in bolded text were used in the base case analysis.

Table 115 The effect on the financial implications of alternative NAAT item fees

	2015	2016	2017	2018	2019
NAAT fee: $28.65					
Total cost of NAAT for TB	$538,276	$562,973	$587,640	$612,336	$637,004
Total cost of NAAT (inc. NAAT for NTM)	$1,759,496	$1,840,223	$1,920,855	$2,001,581	$2,082,214
NAAT fee: $40.00					
Total cost of NAAT for TB	$751,520	$786,000	$820,440	$854,920	$889,360
Total cost of NAAT (inc. NAAT for NTM)	$1,972,740	$2,063,250	$2,153,655	$2,244,165	$2,334,570
NAAT fee: $70.00					
Total cost of NAAT for TB	$1,315,160	$1,375,500	$1,435,770	$1,496,110	$1,556,380
Total cost of NAAT (inc. NAAT for NTM)	$2,536,380	$2,652,750	$2,768,985	$2,885,355	$3,001,590
NAAT fee: $82.00					
Total cost of NAAT for TB	$1,540,616	$1,611,300	$1,681,902	$1,752,586	$1,823,188
Total cost of NAAT (inc. NAAT for NTM)	$2,761,836	$2,888,550	$3,015,117	$3,141,831	$3,268,398
NAAT fee: $100.50					
Total cost of NAAT for TB	$1,888,194	$1,974,825	$2,061,356	$2,147,987	$2,234,517
Total cost of NAAT (inc. NAAT for NTM)	$3,109,414	$3,252,075	$3,394,571	$3,537,232	$3,679,727
NAAT fee: $130.00					
Total cost of NAAT for TB	$2,442,440	$2,554,500	$2,666,430	$2,778,490	$2,890,420
Total cost of NAAT (inc. NAAT for NTM)	$3,663,660	$3,831,750	$3,999,645	$4,167,735	$4,335,630
NAAT fee: $200.00					
Total cost of NAAT for TB	$3,757,600	$3,930,000	$4,102,200	$4,274,600	$4,446,800
Total cost of NAAT (inc. NAAT for NTM)	$4,978,820	$5,207,250	$5,435,415	$5,663,845	$5,892,010

Note: Figures in bolded text were used in the base-case financial analysis.

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