Published in final edited form as:

Int J Tuberc Lung Dis. 2015 November ; 19(11): 1339–1347. doi:10.5588/ijtld.15.0209.

Diagnostic accuracy of nucleic acid amplification tests in urine for pulmonary tuberculosis: a meta-analysis

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Abstract

OBJECTIVE—To determine the diagnostic accuracy of tuberculosis (TB) nucleic acid amplification tests (NAATs) in urine samples for individuals with active pulmonary tuberculosis (PTB).

DESIGN—Systematic review and meta-analysis. Electronic databases and reference lists were searched without age or setting restrictions up to May 2015. Eligible articles examined *Mycobacterium tuberculosis* NAATs in urine samples for PTB diagnosis in patients with sputum culture as the reference standard, and reported sufficient data to separately calculate sensitivity or specificity.

RESULTS—Eight studies, including 1212 participants from seven countries with a mean age ranging from 28 to 48 years, were included. Polymerase chain reaction (PCR) with insertion sequence (IS) *6110*, *rpoB* or *cfp32/hf6* as gene targets was used for NAATs. The pooled sensitivity and specificity was respectively 0.55 (95%CI 0.36–0.72) and 0.94 (95%CI 0.78–0.99), with slightly higher sensitivity in human immunodeficiency virus positive individuals, at 0.59 (95%CI 0.20–0.89). Sensitivity was higher in sputum microscopy-positive than -negative individuals. Storage temperatures below –70°C, centrifuge speed <5000 rpm and IS6110 increased sensitivity on meta-regression.

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Conflicts of interest: none declared.

CONCLUSIONS—Urine *M. tuberculosis* PCR for active PTB diagnosis had high specificity but modest sensitivity (55%). Optimizing specimen handling, gene targets or PCR techniques may improve diagnostic accuracy. Reproducibility data are needed.

Keywords

NAAT; PCR; systematic review; meta-regression

In 2013, it was estimated that there were 9 million incident tuberculosis (TB) cases globally, 3.3 million of whom were not diagnosed.¹ Urine nucleic acid amplification tests (NAATs) have potential for use in the diagnosis of pulmonary tuberculosis (PTB), the most common form of TB worldwide.^{2–4} The relative simplicity of urine collection could be ideal to address diagnostic challenges of active PTB,⁵ and could be exploited in point-of-care (POC) testing of active PTB at all levels of health care, and potentially in households and the community.^{6,7} In addition to being safer to handle, normal urine production yields frequent and relatively large amounts, making urine specimens convenient to obtain and easier to test in comparison to sputum. Children could benefit the most from urine NAATs for PTB diagnosis due to challenges in obtaining sputum samples, particularly from young children who cannot expectorate, and the paucibacillary nature of their disease.^{5,8}

With the advancement of molecular techniques and wider use of the NAAT Xpert[®] MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) in many high TB endemic countries, exploration of alternative samples such as urine for TB diagnosis is increasing due to its ease of collection and potential for decentralized implementation.^{9,10} Cell-free nucleic acids released from dying human cells and *Mycobacterium tuberculosis* microorganisms may be filtered through the kidneys, resulting in the detection of trans-renal DNA fragments in urine.⁶ Although the exact pathophysiological mechanisms underlying the detection of mycobacterial DNA in the urine of patients with active PTB are not well understood, it has high potential relevance for clinical practice.

The objective of this systematic review and meta-analysis was to determine the diagnostic accuracy of *M. tuberculosis* NAATs in urine samples for active PTB compared to sputum culture as the reference standard.

MATERIALS AND METHODS

A formal review protocol was designed prior to analysis. Age, race and human immunodeficiency virus (HIV) status population characteristics were considered a priori as factors for sub-group analysis. Specimen handling techniques and characteristics of the NAAT technology were also considered as covariates in the analysis a priori. Disease severity as evidenced by smear status or presence of cavitation on chest radiography (CXR) were analyzed post hoc.

Eligibility criteria

As is standard in the field of meta-analysis, we applied the PICOTS (population, intervention, comparator, outcome, timing, setting) framework to define the research question, and inclusion and exclusion criteria.¹¹ Articles were considered eligible for

inclusion if they examined NAATs targeting *M. tuberculosis* in urine samples to detect active PTB diagnosis where sputum *M. tuberculosis* culture was the reference standard. We required full texts to be in English and have reported sufficient data to separately calculate sensitivity and specificity. We excluded reviews and case reports. There were no restrictions on population age, study dates, timing and setting.

Information sources, search and study selection

The first author systematically searched the MEDLINE, EMBASE, Web of Science, Scopus, CINAHL (Cumulative Index to Nursing and Allied Health Literature), CABDirect, and the Cochrane Library databases and the World Health Organization Global Health Library to identify studies evaluating urinary TB NAAT use for PTB diagnosis published up to 18 May 2015. The search strategy used was: ‘((TB OR tuberculosis OR *Mycobacterium tuberculosis* OR *M.tb* OR pulmonary tuberculosis OR PTB) AND urine) AND nucleic acid amplification test’.

We identified duplicates after confirming study sites and/or periods and contacting authors for clarification if needed. Where this was not possible or uncertainty persisted, only the publication reporting on the largest samples was included. We screened abstracts for eligibility, retrieved full texts to confirm eligibility and extracted data for quantitative synthesis.

Data collection process

The first author independently extracted data detailed in the Appendix* from eligible articles using a standardized tool. Study outcomes were used to calculate sensitivity and specificity where sputum culture was available for patients with and without active PTB, and for patients with active PTB vs. healthy controls.

We assessed study methodological quality and risk of bias using the Quality Assessment Tool for Diagnostic Accuracy Studies (QUADAS-2) tool,¹² a standard tool with four domains: patient selection, index test, reference standard and flow and timing. All four domains were assessed to determine risk of bias and the first three to determine applicability concerns.

Synthesis of results, risk of bias across studies and additional analyses

For each study, we classified TB NAAT in urine samples as true-positives (TP), false-negatives (FN), false-positives (FP) and true-negatives (TN) as compared with sputum *M. tuberculosis* culture as the gold standard. We then calculated the sensitivity (TP/[TP+FN]) and the specificity (TN/[FP+TN]) for each study and presented our findings as forest plots using the ‘midas.ado’ program in Stata, version 13 (Stata-Corp, College Station, TX, USA).

For analyses including four or more studies, the average accuracy of TB NAAT in urine samples was estimated using a hierarchical summary receiver operating characteristic (HSROC) curve using Stata’s ‘metandi’ command. This model accounts for both within-

*The appendix is available in the online version of this article, at <http://www.ingentaconnect.com/content/ijatld/ijatld/2015/00000019/000000011/art00012>

study (random error) and between-study variability (heterogeneity), and is recommended as the most robust statistical test to determine average sensitivity and specificity provided for diagnostic accuracy.¹³ To date, there are no standardized statistical methods to assess publication bias in diagnostic accuracy meta-analyses.¹³ We also conducted a meta-regression of technological processing covariates using the ‘midas’ command in Stata.

RESULTS

One hundred and eighty two articles were identified through the electronic database search. Three additional records were identified through a manual search of references as well as unindexed articles in the current literature. Duplicates were excluded and, of the remaining 154 abstracts, 29 were eligible for full text assessment. Of these, eight studies^{9,10,14–19} were included for quantitative synthesis as depicted in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow chart (Figure 1).²⁰

Eligible studies were conducted between 1993 and 2013 and included 1212 participants from seven countries. Of the world’s 22 high TB burden countries,¹ South Africa and India were the only countries included. The study designs were mainly cross-sectional or prospective cohort studies. Data specific to HIV could be retrieved from only four studies: these were conducted in Italy, Burkina Faso and South Africa (Table 1 and Figure 2). None of the studies included children; the mean age of the participants ranged from 28 to 48 years. The only NAAT employed in all the studies was the polymerase chain reaction (PCR), and the gene targets included insertion sequence (IS) *6110*, *rpoB* and *cfp32/hf6*.

The highest risk of bias assessed using the QUADAS-2 tool in our analysis was related to patient selection, which was detected in seven out of eight studies (Table 2). Of the 1212 participants, 605 had active PTB and the remainder were either sputum culture-negative or healthy controls.

TB PCR sensitivity could be calculated in all eight studies.^{9,10,14–16,18,19} Specificity for sputum-negative study participants^{9,16–19} and specificity for healthy controls^{14–18} could each be calculated in five studies. Three of these studies included both sputum-negative participants and healthy controls.^{16–18} Two analyses were conducted, one in which both TB cases and controls had a sputum culture, and the second in which healthy individuals (who did not provide sputum) were included as controls. This latter analysis was retained because healthy individuals are not often able to produce sputum.²¹ Both sensitivity and specificity could be calculated in seven studies.^{9,14–19}

There was a high degree of variability in the sensitivity of urine TB PCR ($\bar{P}$ =97%) and specificity with culture-negative controls ($\bar{P}$ =88%), and minimal heterogeneity in the specificity of urine TB PCR with healthy controls ($\bar{P}$ =0%), as shown in the individual study analyses (Figure 2). The average sensitivity and specificity using a random effect model to account for both within-study and between-study variability was estimated at 0.47 (95%CI 0.37–0.64), and the specificity estimated at 0.89 (95%CI 0.51–1.00) when specificity data were derived from patients with negative sputum cultures (Figure 3A). In models using health control data, the average sensitivity was 0.67 (95%CI 0.43–0.84) and specificity 0.99

(95%CI 0.98–1.00) (Figure 3B). The overall analysis yielded sensitivity estimates of 0.55 (95%CI 0.36–0.72) and specificity estimates of 0.94 (95%CI 0.78–0.99) (Figure 3C). The average sensitivity and specificity estimates in the sub-group analysis of sputum microscopy-negative patients were respectively 0.46 (95%CI 0.16–0.79) and 0.98 (95%CI 0.61–0.99) (Figure 4A), 0.53 (95%CI 0.37–0.69) and 0.96 (95%CI 0.55–0.98) in sputum microscopy-positive patients (Figure 4B), and 0.59 (95%CI 0.20–0.89) and 0.98 (95%CI 0.61–0.99) in HIV-positive patients (Figure 4C). Studies in this review lacked explicit documentation of participant's race; only one study reported on CXR findings,¹⁹ and there were no children (age <15 years).

Three covariates were associated with a trend for higher sensitivity, adjusting for a single covariate in meta-regression (Table 3): lower urine storage temperature (sensitivity 0.71 vs. 0.35 for temperature <−70°C vs. ≥−70°C, $P=0.05$); slower centrifuge speed (sensitivity 0.61 vs. 0.19 for <5000 revolutions per minute [rpm] vs. ≥5000 rpm, $P=0.05$); and gene target *IS6110* (sensitivity 0.70 vs. 0.33 for *IS6110* vs. *mpoB*, $P=0.08$). Three covariates were associated with significantly higher specificity: centrifuge speed ≥5000 rpm (specificity 0.99 vs. 0.93 for ≥5000 rpm vs. <5000 rpm $P<0.001$); discarding the urine supernatant (specificity 0.98 vs. 0.92, discarding vs. retaining $P=0.01$); and the gene target *mpoB* (specificity 0.99 vs. 0.94, $P<0.001$). Other covariates, including urine volume, fraction (first-void, mid-stream or total void), duration of storage, additives, e.g., ethylene diamine tetraacetic acid (EDTA) and quality of DNA extraction were either not recorded or were too heterogeneous to analyze using meta-regression.

DISCUSSION

In this systematic review and pooled analysis, which included eight studies involving >1000 participants, urine TB PCR assay sensitivity was <60% and specificity >90% for the detection of active PTB. Urine TB PCR test performance was slightly better in HIV-infected individuals, with a slight increase in sensitivity (59%). The sensitivity of urine TB PCR was also slightly higher in sputum microscopy-positive patients (53%) than in sputum microscopy-negative patients (46%). The pooled specificity of TB PCR in urine for active PTB was high, both in patients with culture-negative sputum and in healthy controls without sputum culture. In one study that included individuals with latent tuberculous infection (LTBI) as controls, the test retained specificity, as would be expected, as systemic TB DNA detection should occur in active TB but not in LTBI.¹⁴

The sensitivity of urine TB PCR was slightly higher in HIV-positive patients (59%), a sub-group of patients who may benefit from this test. Our finding of higher urine TB PCR test sensitivity in HIV-positive than in HIV-negative individuals is consistent with results from urine lipoarabinomannan (LAM) studies for PTB.^{22–25} The overall diagnostic accuracy of urine TB PCR in general, and in HIV sub-groups, was greater than was observed in diagnostic accuracy studies on urine LAM (sensitivity 28–66% and specificity 66–98%),^{23–25} suggesting that mycobacterial PCR in urine may be a better diagnostic test for active PTB than urine LAM. Resistance testing would be an added advantage of urine TB PCR over urine LAM, despite the quadrupled cost, if leveraged on the existing Xpert platform.²⁶ The higher sensitivity of urine TB PCR in sputum microscopy-positive than in

sputum microscopy-negative patients (53% vs. 46%) in this review has also been observed in sputum Xpert, with a sensitivity of 98% in smear-positive patients and 67% in smear-negative patients.²⁷

We noted technical characteristics involving urine specimen handling that reveal opportunities for biotechnological optimization of the assay. Urine TB PCR had high specificity for active PTB, while lower centrifuge speeds, retention of the urine supernatant, and the gene target *IS6110* slightly decreased specificity. However, criteria to enhance sensitivity are more important for this assay. We found trends for increased sensitivity with lower storage temperatures, slower centrifugation speeds, and the use of the gene target *IS6110*. It is postulated that morning urine may contain more DNA than samples collected later in the day, as reported in an unpublished work on human papilloma virus by Vorster et al.²⁸ Although we saw increased sensitivity with morning specimens, this was not statistically significant and should be examined in larger studies. Urine concentration by centrifugation and freezing of samples before DNA extraction has been shown to improve sensitivity of PCR in schistosomiasis.²⁹ DNA detection may be affected by EDTA, regardless of storage temperature.^{30,31} In our review, studies that documented the addition of EDTA to urine samples^{15,18} were too few to analyze on metaregression. It is unclear why centrifuge speeds of 3000–4000 rpm^{9,14–18} would yield higher sensitivity than a speed of 10 000 rpm.¹⁹ Consistent with our findings of increased sensitivity with retention of urine supernatant, previous authors have documented that this contains transrenal DNA, which may be beneficial in increasing detection of TB DNA.^{6,17} The two main gene targets explored in this review include the *M. tuberculosis* complex-specific insertion sequence, *IS6110*, which has been suggested to be the standard tool for TB diagnosis;³² and the gene coding for the β subunit of the bacterial RNA polymerase, *rpoB*, employed in Xpert, which amplifies an *M. tuberculosis*-specific sequence of this gene and detects mutations to determine rifampicin resistance.³³ In the face of an undefined optimal gene target for urine TB NAAT,^{9,10,14,15,17,18} our findings suggest that *rpoB* may be less sensitive than *IS6110*. This calls for further analysis in future studies as the World Health Organization endorsed PCR platform, Xpert, gains wider coverage and is further studied. Data on the reproducibility of the in-house urine PCR assays employed in this review^{14–18} are needed, as these platforms can be highly unreliable.³⁴

TB diagnostics utilizing urine, a relatively simple sample to obtain, would make the diagnosis of PTB readily accessible to patients at virtually any level of health care. Urine PCR optimization would be especially useful in children, for whom sputum collection or gastric aspirates are very challenging and are mainly carried out in secondary and tertiary health facilities.⁵ In a recent systematic review, Detjen et al. reported sub-optimal sensitivity (51–81%) of Xpert in expectorated/induced sputum and gastric lavage samples in children, calling for further research to improve pediatric TB diagnostics.³⁵ However, none of the studies in this review were conducted in children. There are ongoing studies evaluating urine samples in TB patients including children; however, data from these studies are not yet available.

The main limitation of this meta-analysis was the paucity of studies and exclusion of non-English articles. Reviewer bias may have occurred, as the articles were reviewed by a single

reviewer. The studies reviewed were highly biased in patient selection, timing and flow. One approach to mitigate this in future studies is for researchers to follow the standards for the accurate reporting of diagnostic accuracy tests (STARD) criteria.³⁶ Notwithstanding these limitations, we believe that urine TB NAATs could be further optimized to add to the repertoire of TB diagnostics and potentially enhance early detection of active PTB.

CONCLUSION

The pooled sensitivity and specificity of urine TB PCR for active PTB was respectively 55% and 94%, with slightly higher sensitivity in sputum microscopy-positive and HIV-infected individuals. Standardized reporting and adaptation of methodology in future studies may provide more reliable estimates and enhance diagnostic performance, respectively.

Acknowledgments

DM is a recipient of the International AIDS Research Training Program (IARTP) MPH-PhD Implementation Science scholarship funded by the Fogarty International Centre, National Institutes of Health (NIH), Bethesda, MD, USA (1 D43 TW009580). GJS holds an NIH K24 Mentoring Award (K24 HD54314).

APPENDIX

Data extracted included first author name, publication year, language, country, tuberculosis (TB) burden in the region,¹ study design, and study start and stop date. Population characteristics included the mean/median age of the study population, proportion of children, number and proportion of males, setting, number and proportion of inpatients, number and proportion of human immunodeficiency virus (HIV) positive individuals. Characteristics with respect to the collection and handling of urine and sputum samples, including the duration prior to initiating anti-tuberculosis treatment, fraction and time of sample collection, number of samples collected, amount of sample, individual/pooled sample processing, whether the specimen was stored prior to processing and the storage duration and temperature, were extracted. Additional characteristics specific to urine samples obtained included whether the sample was centrifuged and its speed, whether the supernatant was discarded, whether it was incubated, additives and chemicals used to suspend the urine pellet, whether the specimen was stored after processing, type of nucleic acid amplification test employed, the gene target type and other sample processing details. Characteristics specific to sputum specimen extracted included type of sputum culture employed, other sputum tests employed, including microscopy, providing the number and proportion of patients with these tests and other sample processing details provided in the articles.

Table A

PRISMA 2009 checklist

Section/topic	#	Checklist item	Page no
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	1

Section/topic	#	Checklist item	Page no
Abstract			
Structured summary	2	Provide a structured summary, including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number	3 Not registered
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	4
Objectives	4	Provide an explicit statement of questions being addressed with reference to PICOS	4 and 5
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number	5; not available online
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale	4 and 5
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	5
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	5
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6; Appendix 1
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6 and 7
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means)	6
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., $\bar{P}$) for each meta-analysis	6
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies)	7
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified	7
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	7, Figure 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations	7, Table 1

Section/topic	#	Checklist item	Page no
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12)	7, Table 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: 1) simple summary data for each intervention group, 2) effect estimates and confidence intervals, ideally with a forest plot	8, Figures 2 and 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	8 and 9, Figures 2 and 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15)	7, Table 2
Discussion			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., health care providers, users, and policy makers)	9–11
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias)	11
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	12
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	2

PICOS =participants, interventions, comparisons, outcomes, and study design.¹

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Figure 1.

PRISMA flow chart. TB=tuberculosis; EPTB=extra-pulmonary TB; PTB=pulmonary TB; NAAT =nucleic acid amplification test; LAM=lipoarabinomannan; + = positive; - = negative; HIV = human immunodeficiency virus; PRISMA = Preferred Reporting Items for Systematic Reviews and Meta-Analyses.

Figure 2.

Individual study **A**) sensitivity = $TP/(TP+FN)$ where the index test is urine NAAT and the reference is sputum culture;^{7,8,14–19} **B**) specificity = $TN/(TN+FP)$ where the index test is urine NAAT and the reference is sputum culture.^{7,13,16–19}; and **C**) specificity $TN/(TN+FP)$ where the index test is urine NAAT and healthy controls were considered not to have active pulmonary tuberculosis.^{14–18} CI = confidence interval; df=degree of freedom; TP=true-positive; FN=false-negative; NAAT = nucleic acid amplification test; TN = true-negative; FP = false-positive.

Figure 3.

HSROC plots. CI =confidence interval; HSROC =hierarchical summary receiver operator characteristic. ○ = study estimate; ■ = summary point; — HSROC curve; --- =95% CI; - - - =95% prediction region. *Healthy controls.

Figure 4.

Sub-group analysis (HSROC) plots: sputum microscopy and HIV status. *Imputed values.

HIV = human immunodeficiency virus; CI =confidence interval; HSROC =hierarchical summary receiver operator characteristic. ○ = study estimate; ■ = summary curve; point; — =HSROC — — =95%CI; - - - =95% prediction region.

Table 1

Study characteristics

First author, year	Country (TB burden and World Bank Classification)	Study design	Setting	Number of participants (overall sample size)	Active PTB smear-positive %	Mean age years	Male %	HIV %	M. tuberculosis gene target [*]
Acceti, 1999 ¹⁴	Italy (low TB burden, HIC)	Cross-sectional	In-patient	Active PTB: 13 Controls: 143 [†] (156)	NR	28	90	100 44	IS6110
Kafwabulula, 2002 ¹⁵	Zambia (low TB burden, LMIC)	Cross-sectional	Out-patient clinic	Active PTB: 63 Controls: 63 [†] (126)	NR	32	NR	79 0	IS6110 [‡]
Torrea, 2005 ¹⁶	Burkina Faso (low TB burden, LIC)	Prospective	Clinic	Active PTB: 217 Controls: 30 [§] , 55 [†] (405; 331 TB; 74 controls)	210 (97)	36	65	41 40	IS6110
Cannas, 2008 ¹⁷	Italy (low TB burden, HIC)	Cross-sectional	Clinic/hospital	Active PTB: 43 Controls: 10 [§] , 13 [†] (66)	41 (95)	34	70	6 0	IS6110
Gopinath, 2009 ¹⁸	India (high TB burden, LMIC)	Prospective	Hospital	Active PTB: 46 Controls: 35 [§] , 112 [†] (193; 81 TB; 112 controls)	0	48	72	NR NR	cfp32/hf6
Lawn, 2012 ^{19¶}	South Africa (high TB burden, HIC)	Retrospective	Clinic	Active PTB: 84 Controls: 84 [§] (602)	24 (29)	34 [#]	39	100 100	mpoB
Peter, 2012 ⁹	South Africa (high TB burden, UMIC)	Prospective	Hospital	Active PTB: 113 Controls: 62 [§] (281)	59 (52)	35	41	100 100	mpoB
Shenai, 2013 ¹⁰	South Africa (low TB burden, UMIC); South Korea (low TB burden, HIC)	Prospective	Clinic	Active PTB: 26 (44; 35 South Africa; 9 South Korea)	0	NR	NR	0	mpoB

^{*} TB nucleic acid amplification urine tests in all of these studies employed PCR.

[†] Healthy controls

[‡] Githui technique.

[§] Sputum culture-negative.

[¶] The only study in which chest radiography was documented: cavitation documented in 2 (2.5%) active PTB patients.

[#] Median age.

TB=tuberculosis; PTB=pulmonary TB; HIV=human immunodeficiency virus; HIC=high-income country; NR=not recorded; IS=insertion sequence; LMIC=lower-middle-income country; LIC = low-income country; UMIC = upper-middle-income country; PCR = polymerase chain reaction.

Table 2

Study quality assessment (QUADAS-2 tool)

Study	Risk of bias			Flow and timing			Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard		
Aceti ¹⁴	High	High	Low	High	Low	Low	Low		Low
Kafwabulula ¹⁵	High	Low	Low	High	Low	Low	Low		Low
Torrea ¹⁶	High	Low	Low	High	Low	Low	Low		Low
Cannas ¹⁷	High	Unclear	Low	High	Low	Low	Low		Low
Gopinath ¹⁸	High	Unclear	High	High	Low	Low	Low		High
Lawn ¹⁹	High	High	High	High	Low	Low	Low		Low
Peter ⁹	Unclear	Low	Unclear	High	Low	Low	Low		Low
Shenai ¹⁰	Unclear	High	High	Unclear	Low	Low	Low		Low

QUADAS =Quality Assessment Tool for Diagnostic Accuracy Studies.

Table 3Meta-regression of covariates: urine processing and NAAAT technology ($n=7$)^{*}

Parameter	Studies n	Sensitivity % (95%CI)	P value	Specificity % (95%CI)	P value
Sample					
Morning ^{14,16}	3	0.67 (0.38–0.95)	0.20	0.95 (0.81–1.00)	0.13
Spot ^{9,17,18}	3	0.38 (0.13–0.64)		0.95 (0.81–1.00)	
Urine storage temperature [†]					
<–70°C ^{14,15,17,18}	4	0.71 (0.54–0.88)	0.05	0.96 (0.86–1.00)	0.10
≥–70°C ^{9,16,19}	3	0.35 (0.17–0.53)		0.93 (0.77–1.00)	
Centrifuge speed, rpm					
<5000 ^{9,14–18}	6	0.61 (0.46–0.76)	0.05	0.93 (0.82–1.00)	<0.001
≥5000 ¹⁹	1	0.19 (0.03–0.42)		0.99 (0.96–1.00)	
Supernatant					
Retained ^{9,14,15,17,18}	5	0.61 (0.36–0.87)	0.57	0.92 (0.79–1.00)	0.01
Discarded ^{16,19}	2	0.47 (0.08–0.87)		0.98 (0.93–1.00)	
Gene targets [†]					
IS6110 ^{4–17}	4	0.70 (0.48–0.91)	0.08	0.94 (0.84–1.00)	<0.001
<i>rpoB</i> gene target ^{9,19}	2	0.33 (0.04–0.61)		0.99 (0.96–1.00)	

^{*} Adjusting single covariates of urine processing in meta-regression in seven studies: Aceti,¹⁴ Kafwabulula,¹⁵ Torrea,¹⁶ Cannas,¹⁷ Gopinath,¹⁸ Lawn,¹⁹ Peter.⁹[†] Timing of collection of the urine sample and gene targets IS6110 or *rpoB* gene targets was documented/assessed in 6/7 studies.

NAAAT =nucleic acid amplification test; CI =confidence interval; rpm =revolutions per minute; IS =insertion sequence.