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Research Article

Which sample type is better for Xpert MTB/RIF to diagnose adult and pediatric pulmonary tuberculosis?

Mengyuan Lyu[1,2,*], Jian Zhou[2,*], Yuhui Cheng[2], Weelic Chong[3], Kang Wu[2], Teng Fang[2], Tianbo Fu[2] and
Binwu Ying[1]

[1]Department of Laboratory Medicine, West China Hospital, Sichuan University, Chengdu 610041, China; [2]West China Medical School/West China Hospital, Sichuan University,
Chengdu, Sichuan Province 610041, P.R. China; [3]Sidney Kimmel School of Medicine, Thomas Jefferson University, Philadelphia, PA, U.S.A.

Correspondence: Binwu Ying (docbwy@126.com, 3236503013@qq.com)

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Objective: This review aimed to identify proper respiratory-related sample types for adult and pediatric pulmonary tuberculosis (PTB), respectively, by comparing performance of Xpert MTB/RIF when using bronchoalveolar lavage (BAL), induced sputum (IS), expectorated sputum (ES), nasopharyngeal aspirates (NPAs), and gastric aspiration (GA) as sample.
Methods: Articles were searched in Web of Science, PubMed, and Ovid from inception up to 29 June 2020. Pooled sensitivity and specificity were calculated, each with a 95% confidence interval (CI). Quality assessment and heterogeneity evaluation across included studies were performed.
Results: A total of 50 articles were included. The respective sensitivity and specificity were 87% (95% CI: 0.84–0.89), 91% (95% CI: 0.90–0.92) and 95% (95% CI: 0.93–0.97) in the adult BAL group; 90% (95% CI: 0.88–0.91), 98% (95% CI: 0.97–0.98) and 97% (95% CI: 0.95–0.99) in the adult ES group; 86% (95% CI: 0.84–0.89) and 97% (95% CI: 0.96–0.98) in the adult IS group. Xpert MTB/RIF showed the sensitivity and specificity of 14% (95% CI: 0.10–0.19) and 99% (95% CI: 0.97–1.00) in the pediatric ES group; 80% (95% CI: 0.72–0.87) and 94% (95% CI: 0.92–0.95) in the pediatric GA group; 67% (95% CI: 0.62–0.72) and 99% (95% CI: 0.98–0.99) in the pediatric IS group; and 54% (95% CI: 0.43–0.64) and 99% (95% CI: 0.97–0.99) in the pediatric NPA group. The heterogeneity across included studies was deemed acceptable.
Conclusion: Considering diagnostic accuracy, cost and sampling process, ES was a better choice than other sample types for diagnosing adult PTB, especially HIV-associated PTB. GA might be more suitable than other sample types for diagnosing pediatric PTB. The actual choice of sample types should also consider the needs of specific situations.

Introduction

Globally, there were 10 million new tuberculosis (TB) cases and 1.24 million deaths in 2018 alone [1]. A body of studies have confirmed that early diagnosis and treatment can prevent most TB deaths [2–4], and thus excellent diagnostic tools need to be developed. The Xpert MTB/RIF (Cepheid, Sunnyvale, CA, United States) was endorsed as a diagnostic test for use in TB endemic countries by World Health Organization (WHO) in 2010 [5]. A systematic review of Xpert MTB/RIF studies has reported different sensitivities of Xpert MTB/RIF, ranging from 25 to 100% [6]. A study has shown the sensitivity of Xpert MTB/RIF varies with the sample types, samples quality, and bacterial load of samples [7]. Thus, choosing proper sample types is critical to improve the diagnostic performance of Xpert MTB/RIF. Given the high
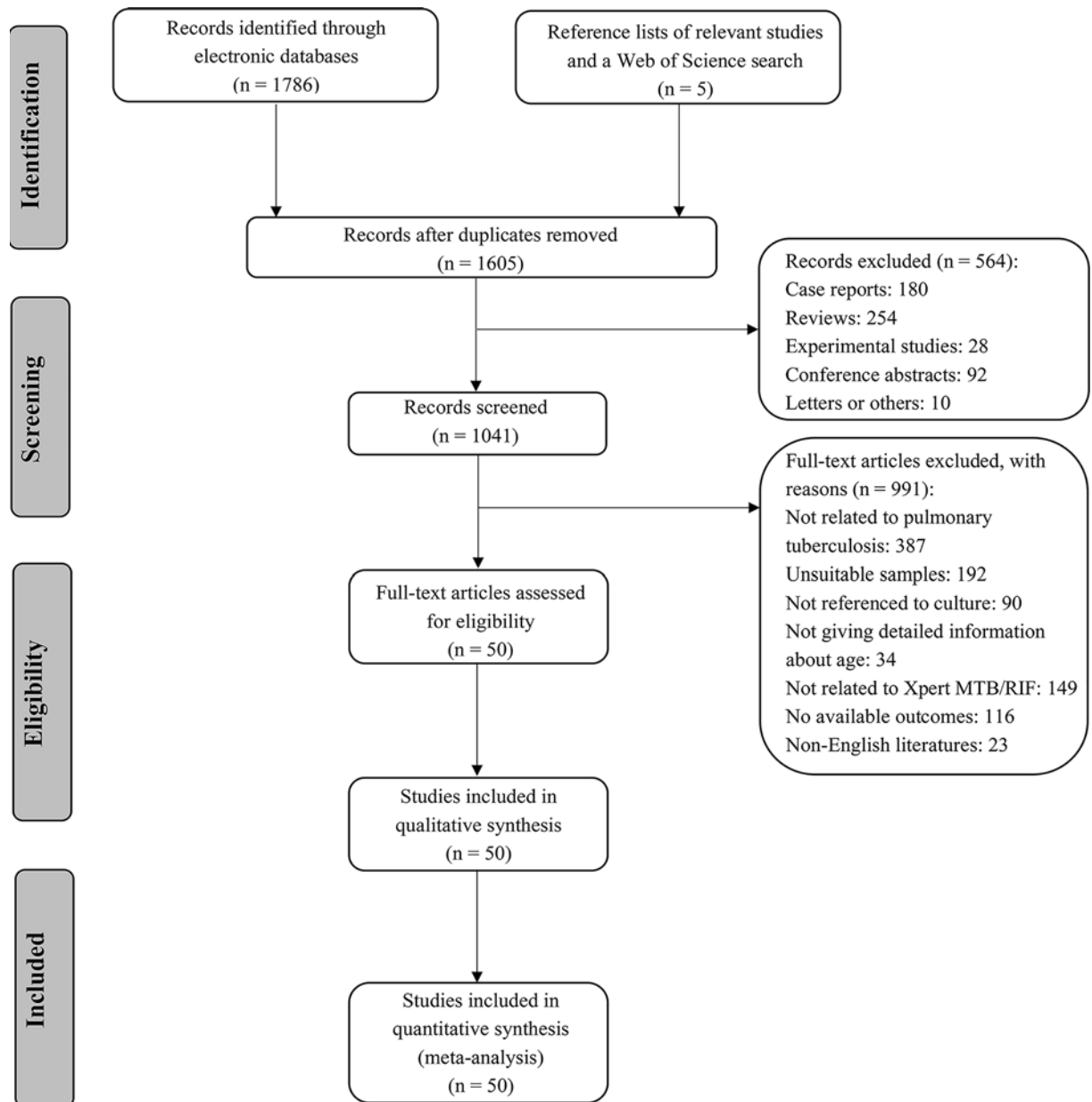
Figure 1. The flow diagram of articles included in this meta-analysis

prevalence of pulmonary TB (PTB) [8], we paid attention to the selection of respiratory-related sample types to better diagnose PTB.

The application of urine and stool for diagnosing PTB have been reported [9,10], however, considering high TB burden areas which are usually economically underdeveloped, high cost of Xpert MTB/RIF and relatively low detection rate in non-respiratory samples [11], respiratory-related sample become the first choice for detecting *Mycobacterium tuberculosis* (MTB) in clinical practice, at least for now. Expectorated sputum (ES), induced sputum (IS), bronchoalveolar lavage (BAL) fluid, gastric aspiration (GA), and nasopharyngeal aspirates (NPAs) are considered suitable samples to detect PTB and are regularly collected in clinical practice. ES is readily available, however, has low bacterial load. IS, sputum induced by the inhalation of hypertonic saline, usually has large sample volume and higher quality than ES [12]. BAL has been widely accepted as the most specific sample type used for accurate and timely diagnosis of lung diseases [13,14]. The WHO supports the collection of BAL to diagnose PTB when possible [15]. However, high cost, invasive sampling and the potential risk of hemorrhage, pneumothorax, laryngospasm and other

adverse reactions limit its application [16]. It is also worth noting that in clinical practice, bronchoscopy and lavage are not considered as feasible in young children due to the potential risk for anesthesia and need of specialized procedural expertise, unless otherwise specified [17]. NPA is sampled after stimulation of the cough reflex via inserting a small feeding catheter into the nasopharynx [18]. Low operational requirements, less invasive sampling, and ready access enable NPA to become an alternative to BAL for pediatric PTB, but not for adult PTB due to significantly different airway microbial composition between NPA and BAL [17]. Young children, particularly who under 5 years, are unable to expectorate sputum and always swallow sputum in their stomach by mistake, thus GA is collected by a nasogastric tube during night in three consecutive mornings to detect MTB for pediatric PTB [19,20]. Nonetheless, MTB is less likely to be detected in GA than smear in adults, and therefore, and thus GA is not regarded as an option for detecting MTB for adult PTB [21]. Enough volume and consecutive samples of GA support repeat tests to improve the rate of detection [22].

Although there are many samples types to choose from, different samples vary greatly in cost, requirement for operators and operating environment, the degree of sampling invasiveness, quality, volume, and others. It is hard to assess which is the best choice for diagnosing PTB. In addition, few studies pay attention to assess the impact of different sample types on the performance of Xpert MTB/RIF when diagnosing PTB. Therefore, we undertook this systematic review and meta-analysis to compare the detection capacity of Xpert MTB/RIF when using ES, IS, and BAL as samples for diagnosing adult PTB, as well as ES, IS, GA, and NPA for diagnosing pediatric PTB. We aimed to summarize and curate reliable evidence for choosing the proper sample types to diagnose PTB patients at different ages.

Materials and methods

Search strategy

We conducted a full search in Web of Science, PubMed, and Ovid on 29 June 2020, with the following terms: 'tuberculosis', *mycobacterium tuberculosis'*, 'TB', 'MTB' AND 'GeneXpert', 'Xpert', 'Xpert MTB/RIF', 'GX' AND 'gastric aspirate', 'gastric aspiration', 'gastric specimen', 'gastric lavage aspiration', 'GA', 'GLA', 'GS', 'bronchoalveolar lavage', 'bronchoalveolar lavage fluid', 'bronchoalveolar washing', 'BAL', 'BALF', 'BW', 'induced sputum', 'IS', 'expectorated sputum', 'ES', 'nasopharyngeal aspirates', 'NPA'. Potential studies were manually identified from reference lists of relevant articles. Any disagreements would be discussed by two independent authors and, if necessary, a third reviewer would arbitrate.

Inclusion and exclusion criteria

Included studies needed to: (i) be cross-sectional studies, cohort studies, or randomized controlled trials; (ii) focus on PTB patients with or without other diseases; (iii) use two diagnostic methods—Xpert MTB/RIF and culture, and take culture as the reference standard; (iv) collect GA, NPA from children, or BAL from adults, or IS or ES from both; (v) give clear information that participants were adults or children. Exclusion criteria were: (i) reviews, case reports, letters, conference abstracts, and animal experiments; (ii) articles not written in English; (iii) articles with incomplete basic data.

Quality assessment

Two independent reviewers assessed the quality of included studies according to the Revised Tool for Quality Assessment of Diagnostic Accuracy Studies (QUADAS)-2 [23]. Both risk of bias and applicability concerns would be evaluated as 'high', 'unclear' or 'low'. Advice of a third reviewer would be referred if necessary.

Data extraction and management

All data were collected and entered into Microsoft Excel version 2016. The basic information mainly comprised the first author, publication year, study population's characteristics, and so on. Diagnostic information included true-positive, false-positive, true-negative, and false-negative. If necessary, we contacted the authors for more essential information, otherwise the trials with incomplete information would be excluded.

Heterogeneity

The threshold effect and non-threshold effect could result in heterogeneity. The heterogeneity caused by the threshold effect could be explored by performing the Spearman correlation coefficient or plotting summary receiver operating characteristic curves (sROCs). The threshold effect would exist if *P*-value of the Spearman correlation coefficient

was less than 0.05 or the points in the plots had a curvilinear (shoulder arm) pattern. If Chi-square *P*-values were less than 0.10, heterogeneity might be caused by a non-threshold effect and a random-effects model would be chosen.

Sensitivity analysis

Sensitivity analysis was performed to determine the robustness of any treatment effect by removing low-quality studies.

Statistical analysis

The diagnostic performance of Xpert MTB/RIF was assessed by calculating the pooled sensitivity, specificity, and area under the curve (AUC), with a 95% confidence interval (CI). The forest plots and sROC were also plotted. We performed statistical analysis with Meta-DiSc 1.4 (XI Cochrane Colloquium, Barcelona, Spain) and Review Manager V.5.3 (The Cochrane Collaboration, Software Update, Oxford, U.K.).

Results
Studies' characteristics

We identified 50 articles: 17 [7,24–39] in the adult BAL group, 14 [35,40–52] in the adult ES group, 7 [35,45,50,53–56] in the adult IS group, 2 [57,58] in the pediatric ES group, 6 [57,59–63] in the pediatric GA group, and 9 [59,64–71] in the pediatric IS group from 1791 studies for the meta-analysis. A total of 2864 BAL samples, 18176 ES samples, and 3876 IS samples were collected from adults. A total of 593 ES samples, 1595 GA samples, 2759 IS samples, and 780 NPA samples were collected from children. More than 80% of all samples were preprocessed, including neutralization, centrifugation, or other processing. The volume of BAL, ES, and IS were 1.0–5 ml, ≥1.0 ml, and 0.5–2.0 ml, respectively. The volume of GA varied from 0.1 ml to more than 5 ml, while 0.5–1.5 ml IS was collected from children.

The flow diagram of articles included in this meta-analysis is presented in Figure 1. The main characteristics of 43 included articles are summarized in Table 1.

Risk of bias

The risk of bias of the included studies is shown in Table 2.

Among 17 studies in the adult BAL group, 5 articles were assessed as low risk and the rest had an unclear risk on the patient selection. For the index test, 13 publications had a low risk and 4 publications had an unclear risk. In the aspect of reference standard and flow and timing, all trials gave clear description about trial details, except for two [27,29]. A total of 14 studies had low concern for applicability, however, the risk of patient selection of 2 studies [37,72] were unclear and that of Ullah et al. [38] was high.

For 14 studies in the adult ES group, 10 studies were assessed as low risk on the patient selection, 3/14 were considered as high risk and the remaining was identified as unclear risk. All of them showed low risk on the index test, reference standard and flow, and timing. Low concern of patient selection and index test appeared in all 14 publications. All studies were evaluated as low risk on reference standard except for two [40,51].

For seven studies in the adult IS group, three articles had low risk of patient selection, three articles had unclear risk of patient selection and only one had high risk of patient selection. Two studies [50,56], which did not state whether the results from two methods were double-blind, assessed as unclear risk on the index test and reference standard. Besides, Luetkemeyer et al. [45] also had an unclear risk on reference standard. All articles in this group were considered as low risk on applicability concerns.

In the pediatric ES group, two studies showed low risk in risk of bias and applicability concerns.

In the pediatric GA group, two articles had low risk of patient selection while others were of unclear risk. Unclear risk of the index test and reference standard only appeared in one publication. Five trials had low risk of flow and timing and applicability concerns, and one [62] was evaluated as high risk for patient selection.

In the pediatric IS group, all studies had low risk of patient selection except two [59,68]. Das et al. [59], LaCourse et al. [64], and Nicol et al. [66] had unclear risk of the index test and reference standard. Five articles had low concern of applicability, while another two studies [64,69] were assessed as high risk of patient selection.

In the pediatric NPA group, two studies showed low risk of bias and applicability concerns.

Heterogeneity

The value of Spearman correlation coefficient showed that the threshold effects did not exist in the adult BAL group (Spearman correlation coefficient: 0.158; *P*=0.727), adult ES group (Spearman correlation coefficient: 0.251;

Table 1 The main characteristics of included trials

Study (year)	Country or Area	Patient recruitment	Sample size	Sample processing	Smear-positive rate (%)	HIV-positive rate (%)	Culture type
Adult BAL[1] group							
Barnard, 2015	South Africa	NA[2]	112	Decontamination and centrifugation	15	NA	MGIT
Gowda, 2018	India	NA	60	Unprocessed	12	7	MGIT
Jo, 2016	South Korea	NA	320	Centrifugation	8	0	MGIT and Ogawa
Khalil, 2015	Pakistan	NA	93	NA	37	NA	LJ[3]
Kilaru, 2019	India	Inpatients and outpatients	56	Decontamination and centrifugation	38	0	MGIT
Ko, 2016	Korea	NA	249	NA	NA	0	MGIT and Ogawa
Lee, 2013	Korea	NA	132	Decontamination and centrifugation	5	0	MGIT and Ogawa
Lu, 2018	China	NA	238	Decontamination	9	NA	MGIT and LJ
Mok, 2016	Singapore	Inpatients and outpatients	158	NA	6	NA	MGIT
Pan, 2018	China	NA	190	Decontamination	19	NA	MGIT
Prakash, 2018	Six countries	Inpatients and outpatients	383	NA	6	NA	MGIT and LJ
Sánchez-Cabral, 2020	Brazil	NA	54	NA	NA	41	MGIT and LJ
Sharma, 2015*	India	NA	131	Decontamination and centrifugation	NA	NA	MGIT and LJ
Theron, 2013	South Africa	NA	154	Centrifugation	10	30	MGIT
To, 2018	China	NA	237	NA	NA	2	NA
Ullah, 2017	Pakistan	NA	98	Decontamination	0	100	NA
Silva, 2019	Brazil	NA	199	NA	NA	NA	MGIT and LJ
Adult ES[4] group							
Al-Darraji, 2013	Malaysia	Prison	125	Decontamination and centrifugation	NA	100	MGIT
Boum, 2016	Uganda	NA	1016	NA	20	66	MGIT
Friedrich, 2011	South Africa	NA	292	Decontamination and centrifugation	97	42	MGIT
Geleta, 2015	Somali	NA	227	Decontamination	26	NA	MGIT and LJ
LaCourse, 2016	Kenya	NA	288	Decontamination	NA	100	MGIT
Luetkemeyer, 2016*	American, Brazil, and Rio de Janeiro	Inpatients and outpatients	992	Unprocessed	15	NA	MGIT and solid media
Madico, 2016	Mbarara and Uganda	Inpatients	261	Decontamination	15	100	MGIT
Pinyopornpanish, 2015	Thailand	NA	57	NA	14	46	MGIT
Rachow, 2011	Tanzania	NA	126	Decontamination	NA	NA	MGIT and LJ
Rasheed, 2019	Pakistan	NA	225	Unprocessed	0	NA	LJ
Rice, 2017*	American	NA	637	Decontamination and centrifugation	30	NA	MGIT and LJ
Sahle, 2019	Ethiopia	Prison	13803	NA	0.1	0.6	MGIT
Sharma, 2015*	India	NA	71	Decontamination and centrifugation	NA	NA	MGIT and LJ
Yeong, 2020	Australia	Inpatients	56	Decontamination	11	0	MGIT
Adult IS[5] group							
Acuna, 2017	Uganda	Inpatients and outpatients	860	Decontamination	89	69	MGIT
Luetkemeyer, 2016*	America and Brazil	NA	324	NA	NA	NA	MGIT and solid media
Mishra, 2020	South Africa	NA	585	Decontamination	12	20	MGIT and LJ
O'Grady, 2012	Zambia	Inpatients	881	Centrifugation	18	71	MGIT
Rice, 2017*	America	NA	653	Decontamination and centrifugation	NA	NA	MGIT and LJ
Sharma, 2015*	India	NA	71	Decontamination and centrifugation	NA	NA	MGIT and LJ
Sohn, 2014	Canada	NA	502	Decontamination	3	2	MGIT

Continued over

Table 1 The main characteristics of included trials (Continued)

Study (year)	Country or Area	Patient recruitment	Sample size	Sample processing	Smear-positive rate (%)	HIV-positive rate (%)	Culture type
Pediatric ES group							
Bates, 2013*	Zambia	Inpatients	142	Decontamination and centrifugation	7	31	MGIT
Reither, 2015	Tanzania and Uganda	NA	451	Decontamination	NA	44	MGIT and LJ
Pediatric GA[6] group							
Bates, 2013*	Zambia	Inpatients	788	Decontamination and centrifugation	4	30	MGIT
Das, 2018*	India and adjoining areas of Nepal	Inpatients and outpatients	106	NA	NA	NA	LJ
Hasan, 2017	Pakistan	NA	49	Decontamination and centrifugation	NA	NA	MGIT
Myo, 2018	Myanmar	NA	231	Decontamination and centrifugation	5	13	LJ
Pang, 2014	China	NA	211	Decontamination and centrifugation	0	NA	MGIT
Sharma 2020	India	Outpatients	210	Decontamination	7	NA	MGIT
Pediatric IS group							
Das, 2018*	India and adjoining areas of Nepal	Inpatients and outpatients	8	Nebulization	NA	NA	LJ
LaCourse, 2014	Malawi	NA	300	NA	0	17	MGIT
Nicol, 2011	South Africa	NA	452	Decontamination and centrifugation	6	24	MGIT
Nicol, 2013	South Africa	NA	115	Decontamination and centrifugation	3	15	MGIT
Nicol, 2018	South Africa	NA	367	Decontamination and centrifugation	NA	19	MGIT and LJ
Sekadde, 2013	Uganda	Mixed	250	Digestion and decontamination	6	42	MGIT and LJ
Togun, 2015	Gambia	Outpatients	487	Digestion, decontamination, and centrifugation	1	0	MGIT and LJ
Zar, 2012*	South Africa	Inpatients	396	Decontamination and centrifugation	5	20	MGIT
Zar, 2013*	South Africa	NA	384	Decontamination and centrifugation	1	8	MGIT
Pediatric NPA[7] group							
Zar, 2012*	South Africa	Inpatients	396	Decontamination and centrifugation	4	20	MGIT
Zar, 2013*	South Africa	NA	384	Decontamination and centrifugation	NA	NA	MGIT

*These studies were divided into at least two groups.
a, BAL.
b, Not available.
c, Löwenstein–Jensen medium.
d, ES.
e, IS.
f, GA.
g, NPAs.

$P=0.387$), adult IS group (Spearman correlation coefficient: 0.321; $P=0.482$), pediatric GA group (Spearman correlation coefficient: -0.257; $P=0.623$), and pediatric IS group (Spearman correlation coefficient: 0.200; $P=0.606$). Besides, the distribution of points in sROC curve also confirmed this. Due to the limited articles in the pediatric ES group and pediatric NPA group, Spearman correlation coefficient and sROC were unavailable.

The value of Chi-square P-values indicted heterogeneity was caused by non-threshold effects in the adult BAL group (Chi-square: 42.33; $P<0.001$), adult ES group (Chi-square: 52.15; $P<0.001$), adult IS group (Chi-square: 18.95; $P=0.004$), pediatric ES group (Chi-square: 13.89; $P=0.002$) and pediatric GA group (Chi-square: 47.45; $P<0.001$). However, non-threshold effects were not detected in the pediatric IS group (Chi-square: 8.40; $P=0.396$), and pediatric

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Table 2 The risk of bias of the included studies

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
Adult BAL[1] group							
Barnard, 2015	unclear	unclear	low	low	low	low	low
Gowda, 2018	unclear	low	low	low	low	low	low
Jo, 2016	unclear	low	low	low	unclear	low	low
Khalil, 2015	unclear	unclear	unclear	unclear	low	low	low
Kilaru, 2019	low	low	low	low	low	low	low
Ko, 2016	unclear	unclear	unclear	unclear	low	low	low
Lee, 2013	unclear	low	low	low	low	low	low
Lu, 2018	low	low	low	low	low	low	low
Mok, 2016	unclear	low	low	low	low	low	low
Pan, 2018	unclear	low	low	low	low	low	low
Prakash, 2018	low	low	low	low	low	low	high
Sánchez-Cabral, 2020	low	low	low	low	low	low	low
Sharma, 2015*	low	low	low	low	low	low	low
Silva, 2019	unclear	unclear	low	low	low	low	low
Theron, 2013	unclear	low	low	low	low	low	low
To, 2018	unclear	low	low	low	unclear	low	low
Ullah, 2017	unclear	low	low	low	high	low	low
Adult ES[2] group							
Al-Darraji, 2013	high	low	low	low	low	low	unclear
Boum, 2016	low	low	low	low	low	low	low
Friedrich, 2011	unclear	low	low	low	low	low	low
Geleta, 2015	low	low	low	low	low	low	low
LaCourse, 2016	high	low	low	low	low	low	low
Luetkemeyer, 2016*	low	low	low	low	low	low	low
Madico, 2016	high	low	low	low	low	low	low
Pinyopornpanish, 2015	low	low	low	low	low	low	low
Rachow, 2011	low	low	low	low	low	low	low
Rasheed, 2019	low	low	low	low	low	low	low
Rice, 2017*	low	low	low	low	low	low	low
Sahle, 2019	low	low	low	low	low	low	unclear
Sharma, 2015*	low	low	low	low	low	low	low
Yeong, 2020	low	low	low	low	low	low	low
Adult IS[3] group							
Acuna, 2017	high	low	low	low	low	low	low
Luetkemeyer, 2016*	unclear	low	unclear	low	low	low	low
Mishra, 2020	low	low	low	low	low	low	low
O'Grady, 2012	unclear	low	low	low	low	low	low
Rice, 2017*	unclear	unclear	unclear	low	low	low	low
Sharma, 2015*	low	low	low	low	low	low	low
Sohn, 2014	low	unclear	unclear	low	low	low	low
Pediatric ES group							
Bates, 2013*	low	low	low	low	low	low	low
Reither, 2015	low	low	low	low	low	low	low
Pediatric GA[4] group							
Bates, 2013*	unclear	low	low	low	low	low	low
Das, 2018*	unclear	unclear	unclear	low	low	low	low
Hasan, 2017	unclear	low	low	low	low	low	low
Myo, 2018	unclear	low	low	low	low	low	low
Pang, 2014	low	low	low	low	high	low	low
Sharma, 2020	low	low	low	low	low	low	low
Pediatric IS group							
Das, 2018*	unclear	unclear	unclear	low	low	low	low

Continued over

Table 2 The risk of bias of the included studies (Continued)

Study	Risk of bias				Applicability concerns		
	Patient selection	Index test	Reference standard	Flow and timing	Patient selection	Index test	Reference standard
LaCourse, 2014	low	unclear	unclear	low	high	low	low
Nicol, 2011	low	low	low	low	low	low	low
Nicol, 2013	low	unclear	unclear	low	low	low	low
Nicol, 2018	low	low	unclear	low	low	low	low
Sekadde, 2013	high	low	low	low	low	low	low
Togun, 2015	low	low	low	low	high	low	low
Zar, 2012*	low	low	low	low	low	low	low
Zar, 2013*	low	low	low	low	low	low	low
Pediatric NPA[5] group							
Zar, 2012*	low	low	low	low	low	low	low
Zar, 2013*	low	low	low	low	low	low	low

*The studies of these authors were divided into at least two groups.
a, BAL.
b, ES.
c, IS.
d, GA.
e, NPAs.
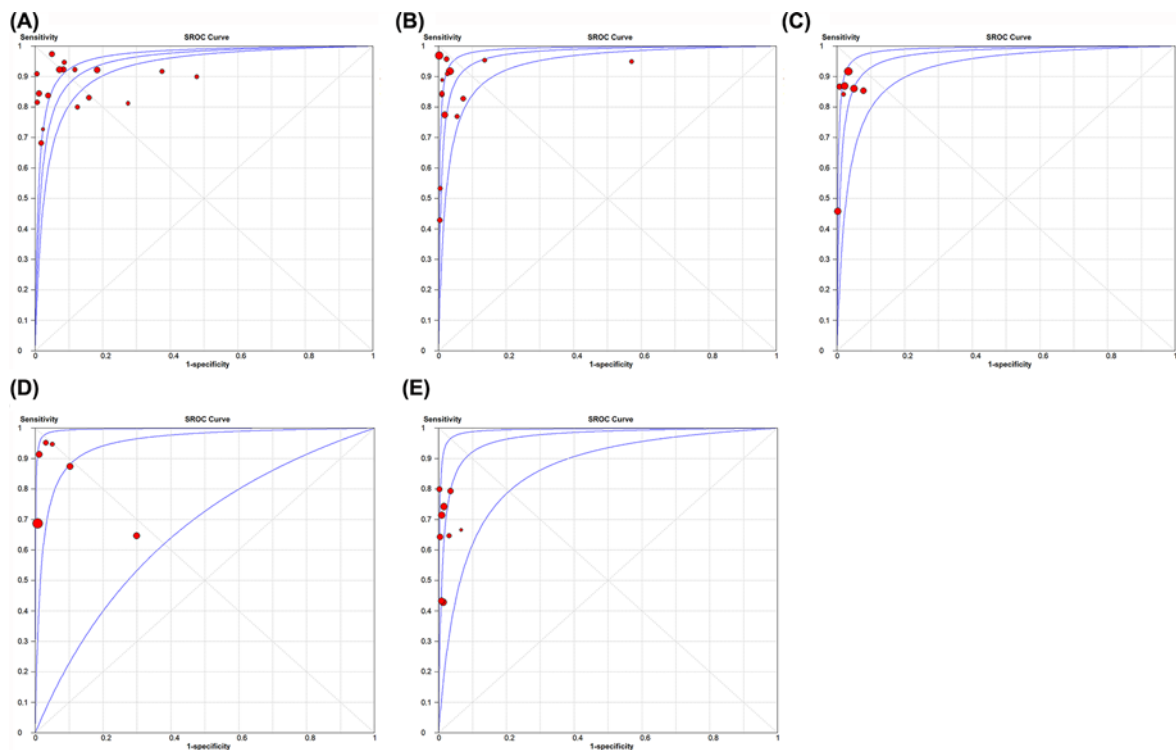
Figure 2. sROC curve for Xpert MTB/RIF of five groups
(**A**) sROC of adult BAL group; (**B**) sROC of adult ES group; (**C**) sROC of adult IS group; (**D**) sROC of pediatric GA group; (**E**) sROC of pediatric IS group.

NPA group (Chi-square: 1.57; $P=0.211$). The sROC curves for Xpert MTB/RIF of five groups (adult BAL group, adult ES group, adult IS group, pediatric GA group, and pediatric IS group) are shown in Figure 2.
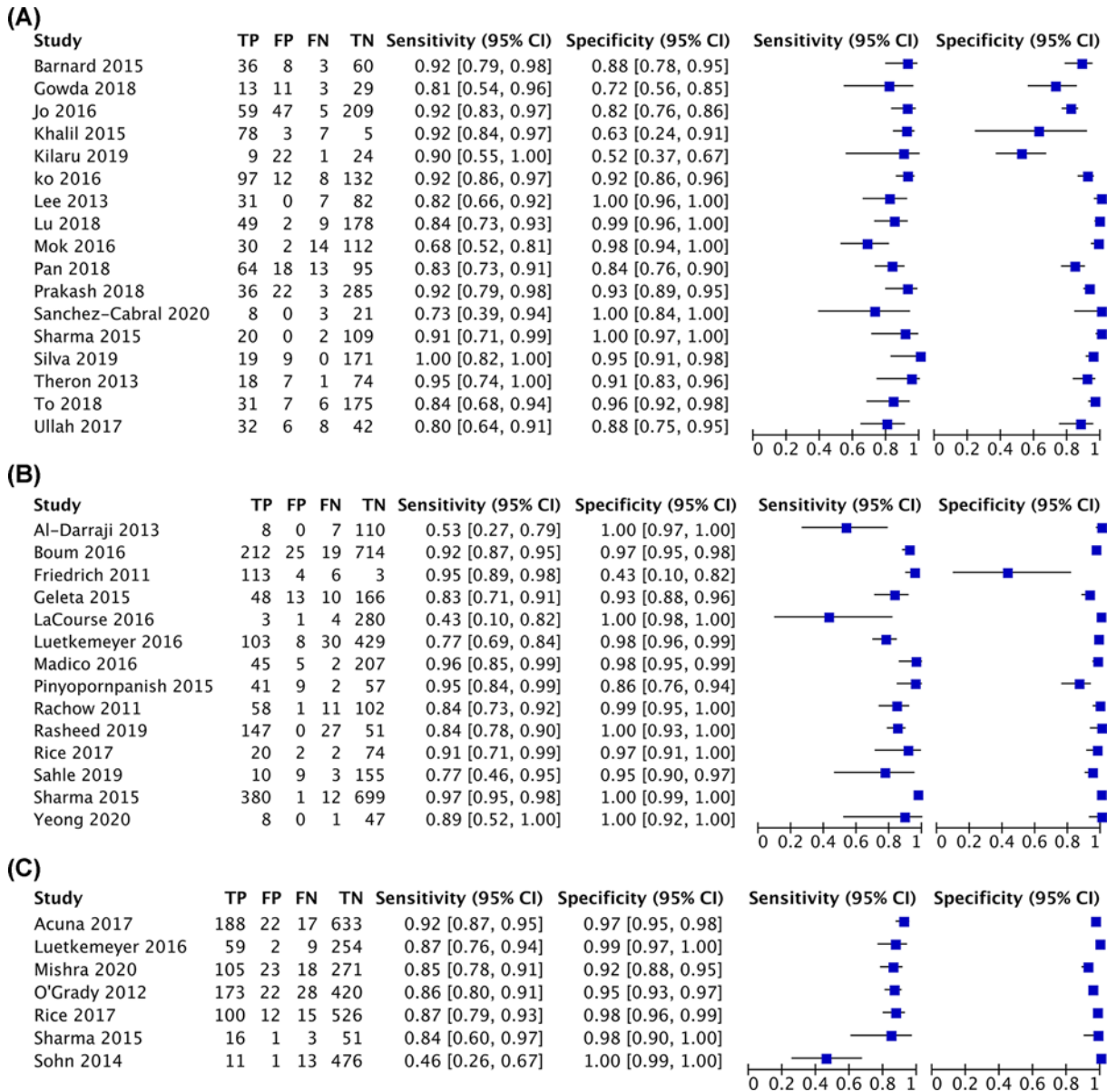
(A)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Barnard 2015	36	8	3	60	0.92 [0.79, 0.98]	0.88 [0.78, 0.95]
Gowda 2018	13	11	3	29	0.81 [0.54, 0.96]	0.72 [0.56, 0.85]
Jo 2016	59	47	5	209	0.92 [0.83, 0.97]	0.82 [0.76, 0.86]
Khalil 2015	78	3	7	5	0.92 [0.84, 0.97]	0.63 [0.24, 0.91]
Kilaru 2019	9	22	1	24	0.90 [0.55, 1.00]	0.52 [0.37, 0.67]
ko 2016	97	12	8	132	0.92 [0.86, 0.97]	0.92 [0.86, 0.96]
Lee 2013	31	0	7	82	0.82 [0.66, 0.92]	1.00 [0.96, 1.00]
Lu 2018	49	2	9	178	0.84 [0.73, 0.93]	0.99 [0.96, 1.00]
Mok 2016	30	2	14	112	0.68 [0.52, 0.81]	0.98 [0.94, 1.00]
Pan 2018	64	18	13	95	0.83 [0.73, 0.91]	0.84 [0.76, 0.90]
Prakash 2018	36	22	3	285	0.92 [0.79, 0.98]	0.93 [0.89, 0.95]
Sanchez–Cabral 2020	8	0	3	21	0.73 [0.39, 0.94]	1.00 [0.84, 1.00]
Sharma 2015	20	0	2	109	0.91 [0.71, 0.99]	1.00 [0.97, 1.00]
Silva 2019	19	9	0	171	1.00 [0.82, 1.00]	0.95 [0.91, 0.98]
Theron 2013	18	7	1	74	0.95 [0.74, 1.00]	0.91 [0.83, 0.96]
To 2018	31	7	6	175	0.84 [0.68, 0.94]	0.96 [0.92, 0.98]
Ullah 2017	32	6	8	42	0.80 [0.64, 0.91]	0.88 [0.75, 0.95]

(B)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Al–Darraji 2013	8	0	7	110	0.53 [0.27, 0.79]	1.00 [0.97, 1.00]
Boum 2016	212	25	19	714	0.92 [0.87, 0.95]	0.97 [0.95, 0.98]
Friedrich 2011	113	4	6	3	0.95 [0.89, 0.98]	0.43 [0.10, 0.82]
Geleta 2015	48	13	10	166	0.83 [0.71, 0.91]	0.93 [0.88, 0.96]
LaCourse 2016	3	1	4	280	0.43 [0.10, 0.82]	1.00 [0.98, 1.00]
Luetkemeyer 2016	103	8	30	429	0.77 [0.69, 0.84]	0.98 [0.96, 0.99]
Madico 2016	45	5	2	207	0.96 [0.85, 0.99]	0.98 [0.95, 0.99]
Pinyopornpanish 2015	41	9	2	57	0.95 [0.84, 0.99]	0.86 [0.76, 0.94]
Rachow 2011	58	1	11	102	0.84 [0.73, 0.92]	0.99 [0.95, 1.00]
Rasheed 2019	147	0	27	51	0.84 [0.78, 0.90]	1.00 [0.93, 1.00]
Rice 2017	20	2	2	74	0.91 [0.71, 0.99]	0.97 [0.91, 1.00]
Sahle 2019	10	9	3	155	0.77 [0.46, 0.95]	0.95 [0.90, 0.97]
Sharma 2015	380	1	12	699	0.97 [0.95, 0.98]	1.00 [0.99, 1.00]
Yeong 2020	8	0	1	47	0.89 [0.52, 1.00]	1.00 [0.92, 1.00]

(C)

Study	TP	FP	FN	TN	Sensitivity (95% CI)	Specificity (95% CI)
Acuna 2017	188	22	17	633	0.92 [0.87, 0.95]	0.97 [0.95, 0.98]
Luetkemeyer 2016	59	2	9	254	0.87 [0.76, 0.94]	0.99 [0.97, 1.00]
Mishra 2020	105	23	18	271	0.85 [0.78, 0.91]	0.92 [0.88, 0.95]
O'Grady 2012	173	22	28	420	0.86 [0.80, 0.91]	0.95 [0.93, 0.97]
Rice 2017	100	12	15	526	0.87 [0.79, 0.93]	0.98 [0.96, 0.99]
Sharma 2015	16	1	3	51	0.84 [0.60, 0.97]	0.98 [0.90, 1.00]
Sohn 2014	11	1	13	476	0.46 [0.26, 0.67]	1.00 [0.99, 1.00]

Figure 3. The summary plots of adult PTB

(**A**) Summary plots of adult BAL group; (**B**) summary plots of adult ES group; (**C**) summary plots of adult IS group. Abbreviations: FN, false-negative; FP, false-positive; TP, true-positive; TN, true-negative.

Results on diagnostic accuracy

The diagnostic performance of Xpert MTB/RIF was calculated, compared with culture. Xpert MTB/RIF showed the pooled sensitivity, pooled specificity, and AUC were 87% (95% CI: 0.84–0.89), 91% (95% CI: 0.90–0.92) and 95% (95% CI: 0.93–0.97) in the adult BAL group; 90% (95% CI: 0.88–0.91), 98% (95% CI: 0.97–0.98) and 97% (95% CI: 0.95–0.99) in the adult ES group; 86% (95% CI: 0.84–0.89), 97% (95% CI: 0.96–0.98) and 98% (95% CI: 0.94–0.99) in the adult IS group; 80% (95% CI: 0.72–0.87), 94% (95% CI: 0.92–0.95) and 95% (95% CI: 0.87–1.00) in the pediatric GA group; 67% (95% CI: 0.62–0.72), 99% (95% CI: 0.98–0.99) and 97% (95% CI: 0.92–1.00) in the pediatric IS group, respectively. The pooled sensitivity and pooled specificity were 14% (95% CI: 0.10–0.19) and 99% (95% CI: 0.97–1.00) in the pediatric ES group and 54% (95% CI: 0.43–0.64) and 99% (95% CI: 0.97–0.99) in the pediatric NPA group, respectively. Due to the limited articles in the pediatric ES group and pediatric NPA group, the AUC was unavailable.

The summary plots of Xpert MTB/RIF for adult and children are presented in Figures 3 and 4, respectively.
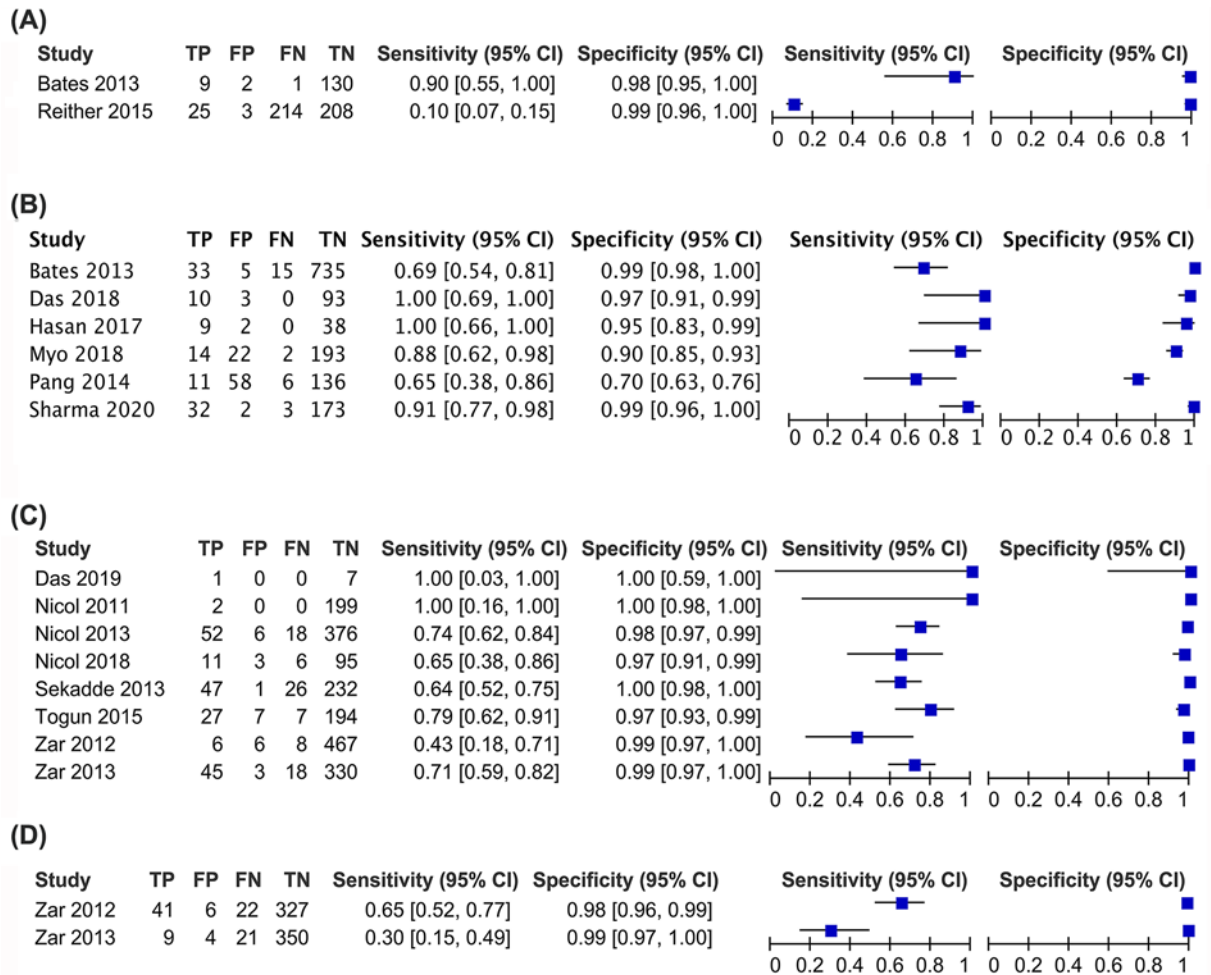
Figure 4. The summary plots of pediatric PTB

(**A**) Summary plots of pediatric ES group; (**B**) summary plots of pediatric GA group; summary plots of pediatric IS group; (**D**) summary plots of pediatric NPA group. Abbreviations: FN, false-negative; FP, false-positive; TN, true-negative; TP, true-positive.

Subgroup analysis was carried out to determine the potential source of heterogeneity among included articles. The detailed information is presented in Supplementary Table S1.

Discussion

Since we only included studies using Xpert MTB/RIF, all comparisons made were between the types of biological samples used. For adults, choosing ES as sample provided better diagnostic performance than BAL or IS. For children, GA showed superior diagnostic capacity to ES, IS, or NPA. Of note, GA also has its own disadvantages including invasive sampling and needing approximately three consecutive days for collecting samples. The actual choice needs to be decided according to specific situations.

It is particularly notorious that microbiology methods (including sputum microscopy and culture), immunological methods [interferon-γ release assay (IGRA) and T-cell spot (T-SPOT)] and molecular methods [polymerase chain reaction (PCR)] are widely applied worldwide before the Xpert MTB/RIF came to market. The celerity and convenience of sputum microscopy make it the most commonly used method in clinical practice, nonetheless low sensitivity (20–40%) is the fatal defect of the sputum microscopy [73]. Culture, as the reference standard for the diagnosis of TB, requires too long detection time and a highly equipped laboratory. Thus, culture is greatly limited in some developing regions typically facing a high TB burden. IGRA and T-SPOT are effective ways to screen latent TB infection. However, inoculating Bacille Calmette–Guérin (BCG), prior infection and inhalation of non-pathogenic

mycobacterium can result in false positivity of IGRA and T-SPOT [74]. Thus, the clinical value of these two diagnostic methods is somewhat discounted in TB endemic areas. Although PCR is a relatively sensitive method to detect MTB, high requirement of operator skills and a laboratory environment also restrict its application. The emergency of Xpert MTB/RIF meets the urgent demand for rapid, simple, integrated, and fully automated detective methods of MTB detection. A myriad of randomized controlled trials (RCTs) have been carried out to assess the diagnostic performance of Xpert MTB/RIF and found the detection rate of MTB is dramatically improved by this novel molecular tool [75]. Apparent benefit brought by Xpert MTB/RIF to PTB patient individually and public health enables this molecular tool to be rapidly accepted and applied all over the world.

However, Xpert MTB/RIF still faces some challenges of inadequate sensitivity when diagnosing smear-negative PTB, pediatric PTB, HIV-associated PTB, and extra-PTB [76,77]. In order to increase diagnostic yield, the Xpert MTB/RIF Ultra (Ultra; Cepheid) appeared. Based on the same platform as the Xpert MTB/RIF, the Ultra harbors some improvements including using two PCR amplification targets (*IS6110* and *IS1081*) and a melt curve analysis [78]. These two changes confer Ultra's lower limit of detection than Xpert MTB/RIF and culture [78]. The following clinical trials have found that improper sample types and poor sample quality still have an adverse influence on the sensitivity of Ultra [79,80]. Obviously, no matter how sensitive the detection method is, choosing proper sample type and ensuring the quality of sample are crucial.

In our systematic review, choosing ES as sample provided better diagnostic performance (sensitivity, specificity, and AUC) than BAL or IS for adult PTB. Since both BAL and IS had a higher bacterial load than ES [81,82], they should theoretically provide better diagnostic performance than ES. However, our study showed opposite results. One reason is the smaller volume of IS, ranging from 0.5 to 2 ml, whereas the volume of ES ranges from 1 ml to more than 2 ml. This inconsistency may also be explained by the ratio of processed samples. The ratio of processed samples in the adult ES group was higher than that in the adult BAL group. In addition, we also found there was a high agreement in diagnosing adult PTB when using IS and BAL as sample. Considering cost, sampling method, acceptance and other factors, IS might be a better choice than BAL to diagnose PTB. Blau et al. [83] gave the interpretation of this results that sampling from one or two parts of lung led to poor repeatability and limited detection rate when taking BAL as sample to diagnose lung infection diseases. Garcia et al. [84] found the gene transcriptional patterns of MTB in BAL and sputum were similar, and additionally, sputum could reflect the physiological state of MTB in the lower airway of PTB patients. Conde et al. [85] conducted a trial and reconfirmed the higher diagnostic yield of taking IS (relative to BAL) as sample to diagnose PTB, in line with our results.

For pediatric PTB, similar specificity and AUC appeared when using IS and GA as sample, nonetheless, Xpert MTB/RIF showed much higher sensitivity when using GA as sample to diagnose pediatric PTB. Although GA is sampled by invasive ways and need approximately three consecutive days for collecting samples, the characteristics of lower probability of contamination, easy sample collection, and sufficient sample volume still make GA become an ideal sample type to increase detection rate for pediatric PTB. Ruiz Jimenez et al. [86] may agree with our results that GA is an excellent sample for diagnosing PTB, and IS should be considered as a supplementary sample type. However, Zar et al. [87] drew an opposite conclusion by conducting a prospective study, with IS for diagnosing PTB and GA as supplementary. The differences may result from different diagnostic tools and the variation in sample collection. The culture, used in their study [87], was unable to detect dead MTB which was caused by the neutralization of GA [88], while Xpert MTB/RIF used in our included studies can detect both dead and live MTB and is not affected by sample neutralization. Furthermore, sample collection, technician skill level, the time from sampling to testing and choice of pre-processing methods also influence finial accuracy [89]. Thus, it is worthy for us to pay attention to the standardization of GA sampling.

Subgroup analysis showed that for adult PTB, superior diagnostic performance appeared in the smear-positive subgroup whether using BAL, ES, or IS as samples. Xpert MTB/RIF showed similar sensitivity whether using smear-positive BAL, smear-positive ES or smear-positive IS, while the highest AUC (90%) was identified when using BAL to diagnose smear-negative PTB. BAL was sampled directly from the lesion region, which was more suitable and reliable for detecting PTB patients with low bacterial load [90,91]. In addition, outperformed capacity of Xpert MTB/RIF appeared when detecting MTB in IS or ES than in BAL for HIV-positive patients. Singer-Leshinsky et al. [92] obtained consistent results with ours that collecting IS from HIV patients may be more beneficial than others. Sample pre-procession steps, the number of samples, the immune status of patient, and the degree of disease severity are the potential confounding factors. More sound evidence is still lacking to support the value of ES in diagnosing HIV-associated adult PTB, which need to be further confirmed.

For pediatric PTB, we reconfirmed the strong association between smear status, bacterial load, and the diagnostic performance of Xpert MTB/RIF. Besides, testing for MTB in IS from children provided a little additional diagnostic yield from the prospective of specificity and AUC for Xpert MTB/RIF when diagnosing smear-negative PTB, however,

Xpert MTB/RIF had higher likelihood to detect MTB in GA from these smear-negative children who really suffered from PTB. Maynard-Smith et al. [6] reported that detecting MTB in GA for sputum-scarce PTB was a good choice. However, Maynard-Smith et al. did not analyze the diagnostic accuracy of Xpert MTB/RIF when detecting MTB in IS from children and make further comparisons. The diagnostic value of GA and IS for smear-negative PTB for children still need to be explored. In the pediatric IS group, subgroup analysis showed that the pooled sensitivity was very heterogeneous between HIV-positive and HIV-negative subgroups and higher sensitivity was in the HIV-positive subgroup. This result was contrary to Connell et al. [93], however, supported by Nicol et al. [65] The smear status, disease severity and samples' pre-processing may affect the results, and a further research should be warranted.

Strengths and limitations

Our study made a rigorous and comprehensive analysis and comparison about the diagnostic performance of Xpert MTB/RIF using different sample types for PTB patients with different ages by including as many studies as possible. Furthermore, we performed reasonable subgroup analysis to identify the source of heterogeneity and potential factors. However, the present study also has some limitations. Limited articles in the pediatric ES group and pediatric NPA group restricted us from further analysis. Some factors including cost, the tolerance of patients and sample contamination rate should be taken into consideration for final choice of diagnostics. Most included articles only used one sample type to analyze the performance of Xpert MTB/RIF, and thus the comparison of different sample types may be influenced by a number of potential confounding factors such as patient characteristics and the process of sampling. Thus, more eligible trials are needed.

Conclusion

When considering diagnostic performance of Xpert MTB/RIF, sampling method and patients' tolerance, using ES as sample might be a better choice to diagnose adult PTB than using BAL and IS. For pediatric PTB, GA was superior to other samples. However, the invasive sampling and relatively long time of collecting samples also should be considered. The actual choice of sample types needs to be decided according to specific situations.

Data Availability
All data generated or analyzed in the present study are contained and presented in this document.

Competing Interests
The authors declare that there are no competing interests associated with the manuscript.

Author Contribution
Mengyuan Lyu designed the present study, conducted literature search, extracted the data, made data analysis and interpretation, drafted and revised the manuscript. Jian Zhou designed the study, conducted literature search, extracted the data, drafted and revised the manuscript. Yuhui Cheng conducted literature search and analyzed the data. Weelic Chong, Kang Wu, Teng Fang and Tianbo Fu extracted data. Binwu Ying gave administrative support. All authors have read and approved the final manuscript.

Abbreviations
AUC, area under the curve; BAL, bronchoalveolar lavage; CI, confidence interval; ES, expectorated sputum; GA, gastric aspiration; IGRA, interferon-γ release assay; IS, induced sputum; MTB, *Mycobacterium tuberculosis*; NPA, nasopharyngeal aspirate; PCR, polymerase chain reaction; PTB, pulmonary tuberculosis; sROC, summary receiver operating characteristic; TB, tuberculosis; T-SPOT, T-cell spot; WHO, World Health Organization.

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