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Original research

TB PCR in BAL and EBUS-TBNA samples for the diagnosis of pulmonary and mediastinal lymph node TB: retrospective TRiBE study

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ABSTRACT

Introduction The role of Xpert Ultra in bronchoalveolar lavage (BAL) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) samples for pulmonary and mediastinal lymph node tuberculosis (TB) remains unclear.

Methods This was a retrospective observational service evaluation at a tertiary TB centre in a low-incidence setting. The diagnostic indices of Xpert Ultra, smear and culture (with cytology for EBUS-TBNA samples) were compared with culture positivity or a composite reference standard of clinical TB diagnosis. Trace readouts, a new category of results for Xpert Ultra indicating low bacillary load, were analysed in two ways as a true positive or true negative result. 282 BAL and 139 EBUS-TBNA samples were included in the analysis.

Results BAL: sensitivity with 95% CI against culture-confirmed pulmonary TB from BAL samples for Xpert Ultra (trace as positive) was 0.91 (0.82 to 0.98), Xpert Ultra (trace as negative) was 0.76 (0.69 to 0.83), smear was 0.38 ($p=0.0009$) and culture was 1.00 (0.91 to 1.00). Specificities for all the tests were ≥ 0.99 (0.98 to 1.00). The addition of smear to Xpert Ultra did not improve the diagnostic accuracy.

EBUS-TBNA: sensitivity against culture-confirmed TB from EBUS-TBNA samples for Xpert Ultra (trace as positive) was 0.71 (0.63 to 0.78), Xpert Ultra (trace as negative) was 0.59 (0.54 to 0.63), smear was 0.12 ($p=0.002$), culture was 1.00 (0.89 to 1.00), cytology was 0.87 (0.76 to 0.98) and rapid on-site evaluation of cytology (ROSE) was 0.92 (0.78 to 1.00). Specificities were 0.99 (0.97 to 1.00), 0.99 (0.97 to 1.00), 1.00 (0.98 to 1.00), 1.00 (0.98 to 1.00), 0.67 (0.67 to 0.68) and 0.42, respectively.

Conclusion Xpert Ultra had a significantly higher sensitivity compared with smear in both BAL and EBUS-TBNA samples. Xpert Ultra had a lower sensitivity compared with culture but comparable specificity with results being available within <24 hours. Trace readings in our low-incidence setting were associated with culture positivity in all BAL samples.

BACKGROUND

Tuberculosis (TB) remains a major global health burden and with COVID-19 disruptions, this has had a significant impact on TB epidemiology and global targets. For the first time in several years, the

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ The clinical utility of Xpert Ultra is well established in sputum but its role in bronchoalveolar lavage (BAL) and endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) samples remains unclear.

WHAT THIS STUDY ADDS

⇒ The study analyses the diagnostic accuracy of Xpert Ultra using BAL and EBUS-TBNA samples and for pulmonary and mediastinal lymph node tuberculosis (TB) in a low-incidence country. In addition, the relevance of trace readings has been evaluated.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Xpert Ultra is a useful rapid diagnostic tool for TB in BAL and EBUS-TBNA samples and has a higher sensitivity compared with smear. Trace readings in our low-incidence setting were likely to reflect TB disease.

number of TB deaths has risen and the rates of TB notification have dropped by 18% from 2019 to 2020.¹ This has led to a significant increase in the diagnostic gap between the incident cases and the number of newly diagnosed cases to 4.1 million,¹ an increase of over a million from 2019. These trends reinforce the ongoing need for a global approach to optimise TB care in order to meet the End TB Strategy.² A crucial area to optimise is the availability of rapid and accurate diagnostic tools.

Current diagnostic methods for TB still rely heavily on smear microscopy and TB culture. Smear microscopy is a reasonable screening tool but lacks in accuracy, and the gold standard of TB culture can often take up to several weeks, especially in paucibacillary specimens such as bronchoalveolar lavage (BAL) or endobronchial ultrasound-guided transbronchial needle aspiration (EBUS-TBNA) samples. With the introduction of molecular methods such as TB PCRs, the diagnostic pathway has significantly improved with rapid results available within hours. The WHO first approved the use of Xpert MTB/RIF (Cepheid, Sunnyvale, USA) in 2010, and in

2013, updated the recommendations to expand its use as the initial diagnostic tests in all persons with signs and symptoms of TB.³ The updated Xpert MTB/RIF Ultra (Xpert Ultra) has been developed in order to improve the diagnostic performance (sensitivity), especially in low bacillary diseases such as in immunosuppressed patients. The new category of results called trace indicates low-burden disease with *Mycobacterium tuberculosis* (MTB) DNA detected through IS6110 and or IS1081 without a signal from the rpoB probes and hence the inability to determine rifampicin resistance. For trace readings, one or both of the probes for the multicopy targets need to be positive with cycle thresholds (Ct) less than 37 cycles and no more than one rpoB probe with a Ct less than 40 cycles.⁴

There are several studies analysing the diagnostic accuracy of Xpert Ultra in pulmonary TB using sputum samples, as well as in extrapulmonary TB.^{5,6} There are no specific studies evaluating the use of Xpert Ultra in bronchial washes or BAL samples, nor dedicated studies for mediastinal lymph node samples obtained via EBUS-TBNA samples in low-incidence high-resource countries. In these settings, patients may have a wider range of diseases and hence the utility of rapid TB molecular diagnostics is unclear.

The aim of this study was to analyse the diagnostic accuracy of Xpert Ultra for the diagnosis of pulmonary TB using BAL samples and for mediastinal lymph node TB using EBUS-TBNA samples. In addition, the relevance of trace readings from Xpert Ultra in a low-incidence setting in the UK was evaluated.

METHODS

Study design and participants

This was a retrospective observational study performed at Imperial College Healthcare National Health Service (NHS) Trust, a tertiary TB centre in London, UK. All consecutive Xpert Ultra results from the North West London Pathology laboratory database from 1 January 2018 to 30 June 2019 were obtained. All patients aged 16 years and older with a differential diagnosis which included TB who underwent a routine bronchoscopy or EBUS-TBNA were included.

Samples were routinely processed as per local hospital guidelines and standard protocols for smear microscopy and culture for microbiological detection of MTB. In addition, immediate cytology with rapid on-site evaluation of cytology (ROSE) and cytological evaluation were reviewed to identify any caseating or non-caseating granulomas for EBUS-TBNA samples.

Xpert Ultra was performed on all samples as part of routine testing. BAL and EBUS-TBNA samples were centrifuged if more than 5 mL were available and the supernatant decanted leaving a 1.5 mL deposit. Sample reagent was added to the specimen and the Cepheid GeneXpert standard operating procedure was followed.⁷ Use of Xpert Ultra for BAL or EBUS-TBNA samples has not been reviewed or approved by any regulatory authority.

BAL samples were cultured for 6 weeks and EBUS-TBNA samples were cultured for 12 weeks with the mycobacteria growth indicator tube (MGIT) system (Becton Dickinson, New

Jersey, USA). When MGIT testing was positive, drug susceptibility testing (DST) was performed in a central laboratory. All culture work was conducted in a class 1 microbiological safety cabinet in containment level 3 facilities.

Clinical information including demographics, comorbidities, risk factors for TB including immunosuppression, previous exposure to TB, previous TB treatment, symptoms, radiology reports and serological data were obtained. BAL and EBUS-TBNA results as well as any other samples available for TB culture such as sputum, pre-bronchoscopy and post-bronchoscopy samples were included if available given their additional value.⁸ The clinical data were reviewed by the treating clinician as part of routine NHS care. A minimum of at least 3 months of follow-up was required for this study. Follow-up data from clinical correspondence reviewing clinical progress, response to any treatment including TB treatment, and change in imaging and other diagnoses were reviewed.

Outcomes

Diagnostic performances (sensitivity, specificity and positive and negative predictive values) of Xpert Ultra, smear and MGIT culture were compared against a composite reference standard using clinical categories attributing to the likely diagnosis of TB (table 1) in BAL samples. For EBUS-TBNA samples, cytological and ROSE samples were evaluated in addition to the above. A composite reference standard was used given the imperfect nature of culture, especially in paucibacillary disease and as used in prior publications.⁹

The final diagnosis of each patient was verified with a composite reference standard by a consensus across a panel of three respiratory consultants with a specialist interest in TB. The panel assessed anonymised clinical data including follow-up data while being blinded to the Xpert Ultra results. Diagnosis of patients was categorised into the following groups: category 1: culture-confirmed TB directly from the BAL or EBUS-TBNA samples (an adjusted category 1 was used for BAL samples which included positive TB cultures from any respiratory samples such as sputum or lung biopsy samples); category 2: highly probable TB with clinical and radiological features suggestive of TB, response to TB treatment and cytological evidence of granulomatous disease if available; category 3: clinical indeterminate diagnosis and category 4: non-TB diagnosis (table 1).

All patients in diagnostic categories 1, 2 and 4 were included in analyses; data for patients in category 3 were reported but not included in the analyses. The new trace readings for Xpert Ultra indicating low-burden disease were dually analysed by incorporating the results as both positive or negative readings. Any incomplete dataset or unprocessed samples for Xpert Ultra, smear or culture were excluded from the study analysis.

The turnaround times for the different TB diagnostic methods from sample collection to the availability of the results were also evaluated.

Table 1 Predefined criteria for case definition and diagnostic categories

Category	Likely diagnosis of TB	Criteria
1	Culture-confirmed TB	Microbiological culture of MTB, and clinical and radiological findings suggestive of TB
2	Highly probable TB	Clinical and radiological features highly suggestive of TB and unlikely to be caused by other diseases, a decision to treat made by a clinician, appropriate response to therapy and cytological evidence (presence of a granuloma) if available
3	Clinically indeterminate diagnosis	Final diagnosis of TB neither highly probable nor reliably excluded
4	Highly unlikely or TB excluded	Other diagnosis made other than TB (eg, sarcoidosis, cancer or lymphoma)

MTB, *Mycobacterium tuberculosis*; TB, tuberculosis.

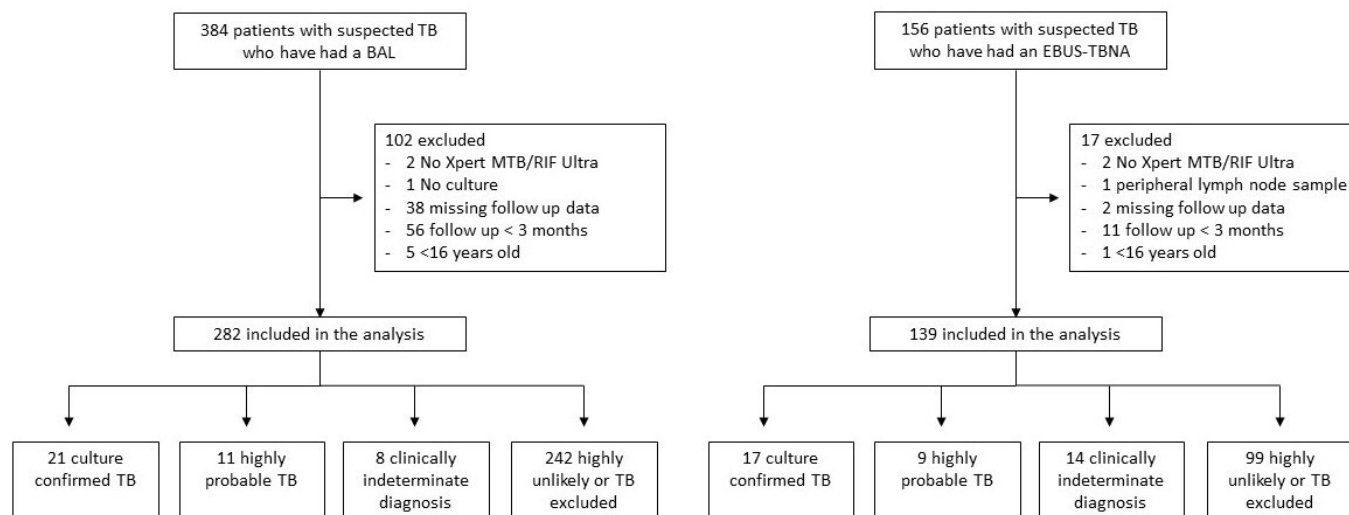


Figure 1 Study flow diagram with $n=348$ BAL samples (left) and $n=156$ EBUS-TBNA samples (right). Flow diagram showing excluded cases and numbers included in the final analysis. The bottom row shows the breakdown of cases according to their clinical diagnostic categories. BAL, bronchoalveolar lavage; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; TB, tuberculosis.

Statistical analysis

Diagnostic sensitivity, specificity, positive and negative predictive values were calculated and 95% CIs were estimated. A statistical comparison of sensitivity using McNemar χ^2 testing with Bonferroni correction for multiple testing to obtain robust p values for the difference in sensitivities between Xpert Ultra and other modalities was performed. Median turnaround times with (IQRs were reported with significance in the turnaround times in comparison with Xpert Ultra analysed using Wilcoxon matched-pairs signed-rank test. All analyses were performed on SPSS and Graphpad Prism V.9.3.0.

RESULTS

Between January 2018 and June 2019, all consecutive 384 BAL samples and 156 EBUS-TBNA samples were reviewed. Of these, 282 BAL and 139 EBUS-TBNA samples had complete datasets with 3 or more months of follow-up and made up the final study population for analysis. A diagram of the study flow is shown in figure 1.

Demographics and the cases in each clinical category are shown in table 2.

BAL samples

The median age of the BAL cohort was 55 years (IQR 41–67), with 60% being male. 23 (8%) had previous TB and 20 (7%) were people living with HIV. One-third of the cohort were on (or recently had) immunosuppressive medications such as prednisolone, chemotherapy or immunotherapy. Just over half the cohort had interferon gamma release assay (IGRA) results available. The median follow-up time for this cohort was 23 months (IQR 9–29).

Of the 282 BAL cases, 21 (7.4%) had a diagnosis of culture-confirmed TB, 11 (3.9%) had highly probable TB, 8 (2.8%) had a clinical indeterminate diagnosis and 242 (85.5%) had a diagnosis of highly unlikely or TB excluded.

For the reference standard of culture-confirmed pulmonary TB directly from BAL samples only (category 1), the diagnostic sensitivity with 95% CI for Xpert Ultra (trace as positive) was 0.91 (0.82 to 0.98), Xpert Ultra (trace as negative) was 0.76 (0.69 to 0.83) with $p=0.083$ for the difference in sensitivities compared with Xpert Ultra (trace as positive), smear was 0.38

($p=0.0009$) and culture was 1.00 (0.91 to 1.00). The specificities for all modalities were ≥ 0.99 (0.98 to 1.00).

When the diagnostic tests were compared against the reference standard of culture-confirmed pulmonary TB from all respiratory samples (adjusted category 1), the sensitivity for Xpert Ultra (trace as positive) was 0.83 (0.77 to 0.90), Xpert Ultra (trace as negative) was 0.71 (0.66 to 0.76) ($p=0.081$), smear was 0.33

Table 2 Demographics table for BAL and EBUS-TBNA samples

	BAL samples	EBUS-TBNA samples
Total number of case	282	139
Age (median with IQR)	55 (41–67)	53 (41–65)
Clinical category		
1: culture-confirmed TB	21 (7.4%)	17 (12.2%)
2: highly probable TB	11 (3.9%)	9 (6.5%)
3: indeterminate	8 (2.8%)	14 (10.1%)
4: highly unlikely TB	242 (85.8%)	99 (71.2%)
Gender		
Male	169 (60%)	84 (60%)
Female	113 (40%)	55 (40%)
Risk factors		
Previous TB	23 (8%)	8 (6%)
HIV	20 (7%)	3 (2%)
Diabetes	42 (15%)	22 (16%)
Chronic kidney disease	43 (15%)	12 (9%)
Immunosuppressive drugs	94 (33%)	26 (19%)
IGRA		
Positive	37 (13%)	35 (25%)
Negative	101 (36%)	51 (37%)
Indeterminate	9 (3%)	4 (3%)
Unknown	135 (48%)	49 (35%)
Ethnicity		
Asian—other	15 (5%)	7 (5%)
Asian—Indian subcontinent	25 (9%)	22 (16%)
Black	34 (12%)	20 (14%)
Mixed	4 (1%)	4 (3%)
Other	108 (38%)	35 (25%)
Unknown	1 (<1%)	3 (2%)
White	95 (34%)	48 (35%)

BAL, bronchoalveolar lavage; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; IGRA, interferon gamma release assay; TB, tuberculosis.

Table 3 Summary of the diagnostic indices for Xpert Ultra, smear and culture in BAL samples

BAL	Xpert Ultra (trace as positive)	Xpert Ultra (trace as negative)	P value*	Smear	P value*	Culture	P value*
Reference standard: culture-positive TB from BAL samples (category 1)							
Positive tests	19/21	16/21		8/21		21/21	
Sensitivity	0.91 (0.82 to 0.98)	0.76 (0.69 to 0.83)	0.083	0.38	0.0009	1.00 (0.91 to 1.00)	—
Specificity	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.00)		0.99 (0.98 to 1.00)		1.00 (0.99 to 1.00)	
PPV	1.00 (0.90 to 1.00)	1.00 (0.88 to 1.00)		0.80 (0.65 to 0.95)		1.00 (0.91 to 1.00)	
NPV	0.99 (0.98 to 1.00)	0.98 (0.97 to 0.99)		0.94 (0.94 to 0.96)		0.99 (0.99 to 1.00)	
Reference standard: culture-positive TB from all respiratory samples (adjusted category 1)							
Positive tests	20/24	17/24		8/24		21/24	
Sensitivity	0.83 (0.77 to 0.90)	0.71 (0.66 to 0.76)	0.081	0.32	0.0005	0.88 (0.80 to 0.95)	0.502
Specificity	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.00)		0.99 (0.98 to 1.00)		1.00 (0.99 to 1.00)	
PPV	1.00 (0.90 to 1.00)	1.00 (0.88 to 1.00)		0.80 (0.65 to 0.95)		1.00 (0.91 to 1.00)	
NPV	0.98 (0.98 to 0.99)	0.97 (0.96 to 0.98)		0.94 (0.93 to 0.94)		0.99 (0.98 to 1.00)	
Reference standard: clinical diagnosis of TB—culture-confirmed TB (category 1) and highly probable TB (category 2)							
Positive tests	20/32	17/32		8/32		21/32	
Sensitivity	0.63 (0.59 to 0.66)	0.53 (0.52 to 0.55)	0.078	0.25	0.0005	0.66 (0.62 to 0.69)	0.564
Specificity	1.00 (0.99 to 1.00)	1.00 (0.99 to 1.00)		0.99 (0.98 to 1.00)		1.00 (0.99 to 1.00)	
PPV	1.00 (0.90 to 1.00)	1.00 (0.88 to 1.00)		0.80 (0.65 to 0.95)		1.00 (0.91 to 1.00)	
NPV	0.95 (0.95 to 0.96)	0.94 (0.93 to 0.95)		0.91 (0.90 to 0.92)		0.96 (0.95 to 0.96)	

*Comparing sensitivity versus Xpert Ultra (trace as positive)—with Bonferroni correction factor. BAL, bronchoalveolar lavage; NPV, negative predictive value; PPV, positive predictive value; TB, tuberculosis.

($p=0.0005$) and culture was 0.88 (0.80 to 0.95) ($p=0.502$). The specificities for all the modalities were ≥ 0.99 (0.98 to 1.00).

When using a reference standard of clinical diagnosis of TB hence using culture-confirmed TB from category 1 in addition to culture-negative but highly probable TB from category 2, the sensitivity for Xpert Ultra (trace as positive) was 0.63 (0.59 to 0.66), Xpert Ultra (trace as negative) was 0.53 (0.52 to 0.55) ($p=0.078$), smear was 0.25 ($p=0.0005$) and culture was 0.66 (0.62 to 0.69) ($p=0.564$). The specificities were all ≥ 0.99 (0.98 to 1.00). The diagnostic indices of Xpert Ultra, smear and culture in BAL samples are summarised in table 3.

When trace readings were incorporated into the positive results, this improved the sensitivity without decreasing the specificity.

When combination of tests were analysed, smear and culture results together had a sensitivity and specificity of 0.88 (0.80 to 0.95) and 0.99 (0.98 to 1.00), respectively, in culture-confirmed

TB for all respiratory samples (online supplemental table 3B). When Xpert Ultra was used in addition, with the trace readings being incorporated as a positive result, the sensitivity increased to 0.92 (0.84 to 0.99) without a drop in the specificity 0.99 (0.98 to 1.00). There was no additional benefit of combining the two rapid diagnostic methods of Xpert Ultra and smear as the diagnostic accuracy did not improve from solely using Xpert Ultra. However, whether Xpert Ultra could replace smear microscopy in the clinical setting is yet to be determined with regard to disease transmission.

For the 21 culture-positive results, the median turnaround time for Xpert Ultra from sample collection was <1 day (IQR 0–1). In comparison with Xpert Ultra, the median turnaround time for smear microscopy was also <1 day (IQR 0–1) ($p=0.627$), for culture was 14 days (IQR 11.0–19.5) ($p<0.0001$) and for DST using whole-genome sequencing was 56 days (IQR 44.0–81.5) ($p<0.0001$). This is summarised in figure 2.

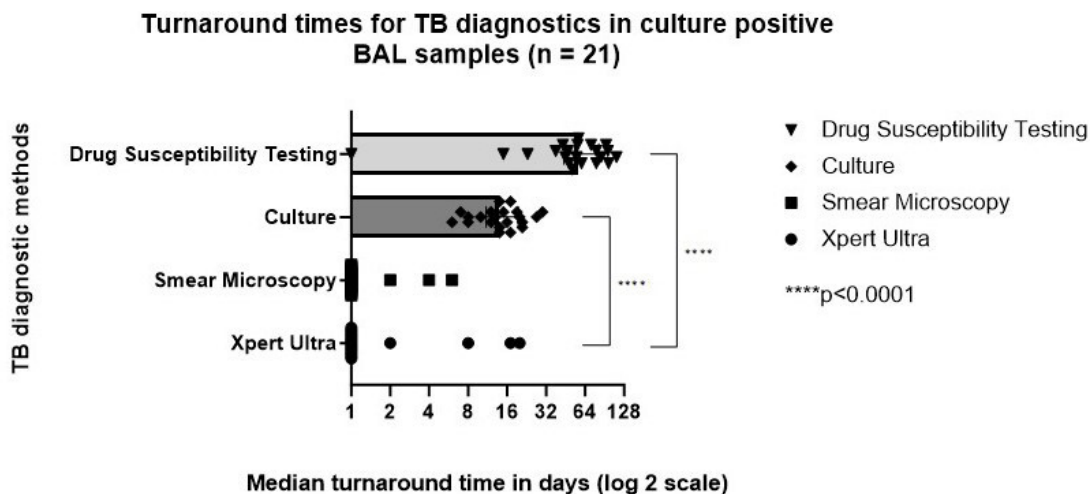


Figure 2 Turnaround times for TB diagnostics in culture-positive bronchoalveolar lavage samples (n=21). The median turnaround time for sample collection for Xpert Ultra and smear was <1 day. For culture, it was 14 days (IQR 11.0–19.5) with a $p<0.0001$ in comparison with Xpert Ultra and for drug susceptibility testing was 56 days (IQR 44.0–81.5) ($p<0.0001$). BAL, bronchoalveolar lavage; TB, tuberculosis.

EBUS-TBNA samples

The median age for the EBUS-TBNA cohort was 53 years (41–65), with the male-to-female ratio being the same as the BAL cohort. Eight (6%) had previous TB and three (2%) had HIV. There was a high proportion of patients taking immunosuppressive drugs (19%). Over one-third of patients were white in ethnicity. The median follow-up period for this cohort was 17 months (IQR 10–23).

Of the 139 EBUS-TBNA samples, 17 (12.2%) had culture-confirmed TB, 9 (6.4%) had highly probable TB, 14 (10.1%) had clinical indeterminate diagnosis and 99 (71.2%) had a diagnosis of highly unlikely or TB excluded.

The diagnostic indices for EBUS-TBNA samples are summarised in table 4. For culture-confirmed TB from EBUS-TBNA samples (category 1), the sensitivity with the 95% CI for Xpert Ultra (trace as positive) was 0.71 (0.63 to 0.78), Xpert Ultra (trace as negative) was 0.59 (0.54 to 0.63) with $p=0.157$ for the difference in sensitivities compared with Xpert Ultra (trace as positive), smear was 0.12 ($p=0.002$), culture was 1.00 (0.89 to 1.00), cytology was 0.87 (0.76 to 0.98) ($p=0.257$) and ROSE was 0.92 (0.78 to 1.00) ($p=0.103$). The specificities were 0.99 (0.97 to 1.00), 0.99 (0.97 to 1.00), 1.00 (0.98 to 1.00), 1.00 (0.98 to 1.00), 0.67 (0.67 to 0.68) and 0.42, respectively.

When using categories 1 and 2 as the reference standard, the sensitivity for Xpert Ultra (trace as positive) was 0.58 (0.55 to 0.61), Xpert Ultra (trace as negative) was 0.39 ($p=0.025$), smear was 0.07 ($p=0.0003$), culture was 0.65 (0.61 to 0.70) ($p=0.480$), cytology was 0.79 (0.73 to 0.86) ($p=0.083$) and ROSE was 0.80 (0.72 to 0.88) ($p=0.020$). The specificities were ≥ 0.99 for Xpert Ultra, smear and culture but were lower at 0.67 and 0.42 for cytology and ROSE, respectively.

Using a combination of tests with rapidly available results, the combined diagnostic yield for ROSE and Xpert Ultra (using trace as a positive reading) increased the sensitivity to 1.00 (0.89 to 1.00) in culture-confirmed cases and 0.89 (0.82 to 0.95) in the combined categories 1 and 2. For routinely available tests (smear, culture and cytology), the combined sensitivity and specificity were 1.00 (0.89 to 1.00) and 0.68 (0.67 to 0.69) with a positive predictive value of 0.35 and negative predictive value of 1.00 (0.97 to 1.00) in culture-positive cases. Using all available test modalities (smear, ROSE, Xpert Ultra, culture, cytology) showed no overall improvements in the diagnostic accuracy compared with the standard tests available in culture-positive cases.

There were five trace readings in EBUS-TBNA samples, of which two were culture positive. Four of the five cases had a positive IGRA and all cases were either immunosuppressed with comorbidities (diabetes or chronic renal disease) or were on immunosuppressive medications. All five cases had no prior TB exposure or prior TB treatment.

For the 17 culture-positive EBUS-TBNA cases, ROSE, Xpert Ultra and smear results were available within 24 hours of sample collection. In comparison with Xpert Ultra, the median turn-around time for cytology was 4 days (IQR 3.0–4.75) ($p<0.0001$), for culture was 17 days (IQR 15–21) ($p<0.0001$) and for DST was 41 days (IQR 36.0–69.5) ($p<0.0001$). Results are shown in figure 3.

DISCUSSION

To date, this is the largest study to analyse the diagnostic accuracy of Xpert Ultra in BAL and EBUS-TBNA samples in a low-burden high-resource setting within a routine clinical setting. For the analysis of BAL samples, the reference standard of culture-confirmed pulmonary TB using a direct comparison of Xpert

Table 4 Summary of diagnostic indices for Xpert Ultra, smear, culture, cytology and ROSE in EBUS-TBNA samples

EBUS	Xpert Ultra (trace as positive)	Xpert Ultra (trace as negative)	P value*	Smear	P value*	Culture	P value*	Cytology	P value*	ROSE	P value*
Reference standard: culture-positive TB diagnosis (category 1)											
Positive tests	12/17	10/17		2/17		17/17		13/15		12/13	
Sensitivity	0.71 (0.63 to 0.78)	0.59 (0.54 to 0.63)	0.157	0.12	0.002	1.00 (0.89 to 1.00)	—	0.87 (0.76 to 0.98)	0.257	0.92 (0.78 to 1.00)	0.103
Specificity	0.99 (0.97 to 1.00)	0.99 (0.97 to 1.00)		1.00 (0.98 to 1.00)		1.00 (0.98 to 1.00)		0.67 (0.67 to 0.68)		0.42	
PPV	0.92 (0.78 to 1.00)	0.91 (0.75 to 1.00)		1.00 (0.02 to 1.00)		1.00 (0.88 to 1.00)		0.29		0.32	
NPV	0.95 (0.93 to 0.97)	0.93 (0.92 to 0.95)		0.87 (0.85 to 0.88)		1.00 (0.98 to 1.00)		0.97 (0.94 to 1.00)		0.95 (0.85 to 1.00)	
Reference standard: clinical diagnosis of TB—culture-confirmed TB (category 1) and highly probable TB (category 2)											
Positive tests	15/26	10/26		2/26		17/26		19/24		16/20	
Sensitivity	0.58 (0.55 to 0.61)	0.39	0.025	0.07	0.0003	0.65 (0.61 to 0.70)	0.480	0.79 (0.73 to 0.86)	0.083	0.80 (0.72 to 0.88)	0.020
Specificity	0.99 (0.97 to 1.00)	0.99 (0.97 to 1.00)		1.00 (0.98 to 1.00)		1.00 (0.98 to 1.00)		0.67 (0.66 to 0.68)		0.42	
PPV	0.94 (0.82 to 1.00)	0.91 (0.75 to 1.07)		1.00 (0.02 to 1.00)		1.00 (0.88 to 1.00)		0.37		0.39	
NPV	0.90 (0.88 to 0.92)	0.86 (0.85 to 0.87)		0.80 (0.79 to 0.82)		0.92 (0.90 to 0.93)		0.93 (0.90 to 0.95)		0.82 (0.75 to 0.89)	

*Comparing sensitivity versus Xpert Ultra (trace as positive)—with Bronchovital correction factor. EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; NPV, negative predictive value; PPV, positive predictive value; ROSE, rapid on-site evaluation of cytology; TB, tuberculosis.

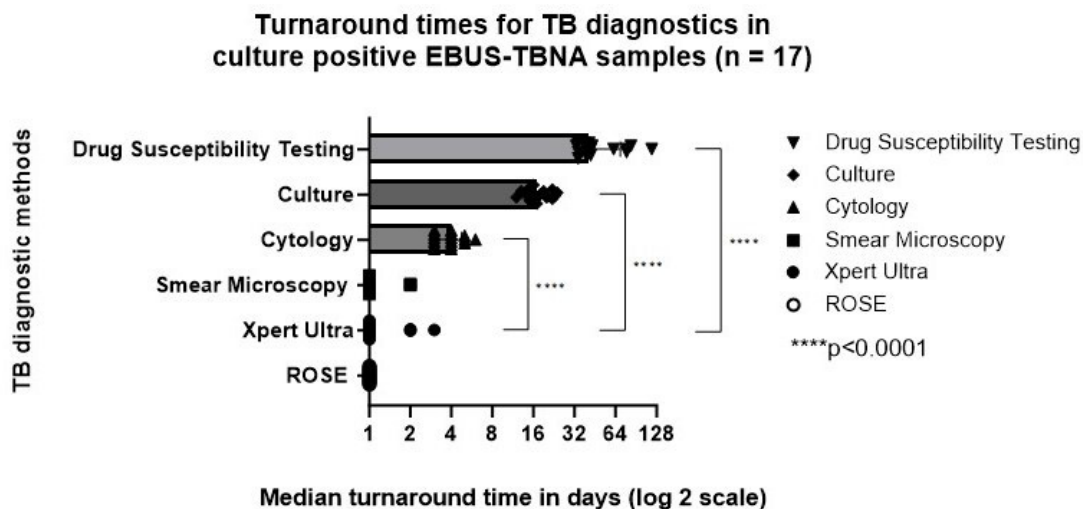


Figure 3 Turnaround times for TB diagnostics in culture-positive EBUS-TBNA samples (n=17). ROSE, smear and Xpert Ultra results were all available within 24 hours of sample collection. Cytology results took 4 days (IQR 3.0–4.75) with $p < 0.0001$ when compared with Xpert Ultra. Culture results took a median of 17 days (IQR 15–21) ($p < 0.0001$) and drug susceptibility results were available after 41 days (IQR 36.0–69.5) ($p < 0.0001$). EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; ROSE, rapid on-site evaluation of cytology; TB, tuberculosis.

Ultra with BAL cultures was used in addition to the adjusted category 1 which analysed culture-confirmed TB from any respiratory samples to reflect the true nature of clinical practice.

Xpert Ultra had a statistically significant higher sensitivity compared with smear microscopy in both BAL and EBUS-TBNA samples in all clinical categories. Xpert Ultra had a slightly lower sensitivity (difference of 0.03–0.09 in BAL and by 0.07–0.29 in EBUS-TBNA samples) when compared with the gold standard of culture when using a reference standard of culture-positive TB (category 1) and clinical diagnosis of TB (categories 1 and 2). However, the specificity for Xpert Ultra and culture was comparable for all groups. In clinical practice, the drop in sensitivity may be acceptable when taking into consideration the significantly faster turnaround time for Xpert Ultra with the addition of rifampicin susceptibilities.

Considering other diagnostic studies, Chien *et al* evaluated the use of Xpert Ultra in bronchial washing fluid in a high-incidence country and showed a sensitivity of 62.5% in culture-confirmed cases and 63% against a composite reference standard.¹⁰ Although the sensitivities of the composite reference standards align, our sensitivity for culture-confirmed cases was higher at 0.83. This may be due to the variation in epidemiology and incidence as other bronchoscopic studies in low-incidence countries have shown a similar sensitivity of 80% using the previous version of Xpert MTB/RIF.¹¹ A systematic review of 19 studies again using the previous Xpert MTB/RIF has also shown a similar sensitivity for bronchoscopic samples.¹² Other studies using Xpert Ultra analysing mixed respiratory samples using a combination of sputum, bronchial washes, lavages and aspirates against culture have shown a sensitivity between 85% and 87%.^{13 14}

There have been no previous studies evaluating the use of Xpert Ultra solely using EBUS-TBNA samples. Our study showed that Xpert Ultra in EBUS-TBNA samples had a sensitivity of 0.71 and a specificity of 0.99 in culture-positive cases. A small study using (any) lymph node aspirates in the context of extrapulmonary TB demonstrated a sensitivity and specificity of 78% and 100% against culture.¹⁵ A study evaluating EBUS-TBNA samples using the older version of Xpert MTB/RIF showed similar diagnostic values to our study, with a sensitivity of 72.6% and specificity of 96.3%.¹⁶ However, there has not been a head-to-head comparison between Xpert MTB/RIF and Xpert Ultra in EBUS-TBNA

samples but it does confirm the consistent performance of the GeneXpert PCR test in EBUS-TBNA samples.

In our study, there were eight trace readings: three in BAL samples which were all culture positive and five in EBUS-TBNA samples, of which two were culture negative and could be explained by the paucibacillary nature of mediastinal lymph node TB. None of the cases with trace readings had previous TB. For both BAL and EBUS-TBNA samples, using trace results as a true positive resulted in higher diagnostic accuracy compared with using trace results as a negative reading without compromise to the specificity.

With regard to optimising diagnostic tests and considering diagnostic pathways, a combination of different tests analysing their sensitivities and specificities was performed (online supplemental tables 3B and 4B), taking into account the speed of the turnaround times and routinely available tests. In BAL samples, the addition of smear to Xpert Ultra did not improve the diagnostic accuracy compared with Xpert Ultra alone. Although the addition of Xpert Ultra in EBUS-TBNA samples to the routinely available tests of smear, culture and cytology did not add to the diagnostic accuracy in culture-positive cases, the rapidity of available results is likely to affect treatment choice and initiation. The use of ROSE and Xpert Ultra in combination had a high specificity and sensitivity with no added value with the addition of the smear result. Culture would still be required for microbiological confirmation and additional drug susceptibilities. This may change in the future with the availability and validation of second-line DST using PCR methods, and next-generation or whole-genome sequencing directly from samples.^{17–21} Although cytology had a lower sensitivity in comparison with ROSE and a low specificity (table 4), this modality is essential for establishing other diagnoses such as malignancies and hence is useful for EBUS-TBNA procedures. As for smear microscopy, the low sensitivity and additional laboratory skills required raise the question of its current role in TB diagnostics. With Xpert Ultra having higher sensitivity, similar specificity, turnaround times <24 hours and the ability to detect rifampicin susceptibility, this could potentially replace smear microscopy (and save a precious portion for culture in the case of EBUS-TBNA samples).

Summary:

- Xpert Ultra provides an early diagnostic tool.
- Xpert Ultra has a significantly higher sensitivity and is clinically more useful than smear.
- Xpert Ultra has a high specificity of at least 0.99 in both BAL and EBUS-TBNA samples in all clinical categories.
- Trace readings in both BAL and EBUS-TBNA samples should be considered as true positive results in cases without previous TB.
- In BAL samples, smear did not add any diagnostic benefit to Xpert Ultra.
- In EBUS-TBNA samples, Xpert Ultra in combination with ROSE increased the sensitivity to 1.00 (95% CI 0.89-1.00) in culture positive and 0.89 (95% CI 0.82-0.95) in clinical diagnosis of TB category.
- Xpert Ultra should be used as a rule in but not rule out test.
- Optimal combination: Xpert Ultra, ROSE, culture and cytology (for TB and non-TB diagnoses).

Figure 4 Summary of key results from this study. BAL, bronchoalveolar lavage; EBUS-TBNA, endobronchial ultrasound-guided transbronchial needle aspiration; ROSE, rapid on-site evaluation of cytology; TB, tuberculosis.

A key strength in this study was the use of a clinical category attributing the likelihood of TB determined by a panel of expert clinicians, reflecting real-life clinical practice in a low-burden high-resource setting. Another strength was the duration of the follow-up for both BAL and EBUS-TBNA cases which allowed confirmation of the initial diagnosis.

This study had several limitations beyond its retrospective nature. Only adults were studied and it is unclear if these data are applicable to children where disease is more paucibacillary. This was a single-centre study in London with a high proportion of immunosuppressed cases and patient demographics may differ elsewhere. There were also no cases with previous TB with trace readings and hence there is a need for a prospective multi-centre study to analyse the diagnostic accuracy of Xpert Ultra in this setting.

In summary, our study demonstrates that Xpert Ultra is a useful rapid diagnostic tool for TB in BAL and EBUS-TBNA samples. Trace readings in this low-incidence setting were also likely to reflect TB disease (figure 4).

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