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Three Year Evaluation of Xpert MTB/RIF in a Low Prevalence Tuberculosis Setting: a Scottish Perspective

Running title: An Evaluation of Xpert MTB/RIF in Scotland

Benjamin J. Parcell[a], Anna Jarchow-MacDonald[b], Amie-Louise Seagar[c], Ian Laurenson[c], Gordon J. Prescott[d] , Michael Lockhart[b].

a) **Aberdeen Royal Infirmary, Aberdeen, United Kingdom, AB25 2ZN**
   b.parcell@nhs.net
b) **Ninewells Hospital and Medical School, Dundee, United Kingdom, DD1 9SY**
   anna.jarchow-macdonald@nhs.net
   michael.lockhart@nhs.net
c) **Scottish Mycobacteria Reference Laboratory SMRL- Royal Infirmary of Edinburgh, 51 Little France Crescent, Old Dalkeith Road, Edinburgh, United Kingdom, EH16 4SA**
   Louise.Seagar@nhslothian.scot.nhs.uk
   Ian.Laurenson@nhslothian.scot.nhs.uk
d) **The Institute of Applied Health Sciences, University of Aberdeen, Aberdeen, United Kingdom**
   gordon.prescott@abdn.ac.uk

Corresponding author:

Benjamin J. Parcell

Consultant in Medical Microbiology & Infection Prevention and Control

Medical Microbiology

Aberdeen Royal Infirmary

Foresterhill

Aberdeen AB25 2ZN

b.parcell@nhs.net

+44 1224 554078

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Abstract

Objectives

Xpert MTB/RIF (Cepheid) is a rapid molecular assay shown to be sensitive and specific for pulmonary tuberculosis (TB) diagnosis in highly endemic countries. We evaluated its diagnostic performance in a low TB prevalence setting, examined rifampicin resistance detection and quantitative capabilities predicting graded auramine microscopy and time to positivity (TTP) of culture.

Methods

Xpert MTB/RIF was used to test respiratory samples over a 3 year period. Samples underwent graded auramine microscopy, solid/ liquid culture, in-house IS*6110* real-time PCR, and GenoType MTBDRplus (HAIN Lifescience) to determine rifampicin and/or isoniazid resistance.

Results

A total of 2103 Xpert MTB/RIF tests were performed. Compared to culture sensitivity was 95.8%, specificity 99.5%, positive predictive value (PPV) 82.1%, and negative predictive value (NPV) 99.9%. A positive correlation was found between auramine microscopy grade and Xpert MTB/RIF assay load. We found a clear reduction in the median TTP as Xpert MTB/RIF assay load increased. Rifampicin resistance was detected.

Conclusions

Xpert MTB/RIF was rapid and accurate in diagnosing pulmonary TB in a low prevalence area. Rapid results will influence infection prevention and control and treatment measures. The excellent NPV obtained suggests further work should be carried out to assess its role in replacing microscopy.

Abstract Word Count 198

Introduction

Diagnosing tuberculosis (TB) can be problematic as patients may present with a wide range of symptoms which may not be specific. In addition, the sensitivity of microscopy and smear positivity in respiratory TB ranges from 57-81%, potentially leading to misdiagnosis.[1] TB culture is the gold standard for diagnosing TB and allows drug susceptibilities to be tested. There have been developments in rapid automated mycobacterial liquid culture systems and time to detection of growth of mycobacterial species can be shortened significantly.[2] Even with these advances there could be delays in diagnosis, leading to later initiation of appropriate therapy and implementation of infection prevention and control measures.

Xpert MTB/RIF (Cepheid) is a rapid, direct molecular test for the diagnosis of pulmonary TB and detection of rifampicin (RIF) resistance, which is a marker of multidrug resistant TB (MDRTB).[3] It has been endorsed by the World Health Organisation (WHO) and extensive evaluation has found it to be sensitive and specific for pulmonary TB diagnosis and detection of RIF resistance in high endemic countries for suspected cases of MDRTB.[4] Xpert MTB/RIF has lower sensitivity in HIV-associated TB.[5] There is however, considerably less data on the use of Xpert MTB/RIF in low prevalence countries despite increased use.[6] Recently a study to examine the use of Xpert MTB/RIF versus AFB smear and culture to identify pulmonary TB found that the diagnostic performance of Xpert MTB/RIF in the United States was similar to higher TB prevalence sites in Brazil and South Africa.[7]

Scotland's TB incidence was 6.5 cases per 100,000 population in 2014 with low rates of MDR-TB (around 0.9%).[8] In that year, Scotland had an estimated rate of 1.51 diagnosed HIV-infected persons per 1000 population in adults aged 15-59 years (Glenn Codere, Health Protection Scotland, Personal Communication 14 December 2016). The UK National Institute for Health and Care Excellence (NICE) guidance recommends rapid diagnostic nucleic acid amplification tests for diagnosing pulmonary (including laryngeal) TB in adults if there is clinical suspicion of TB disease and the person has HIV or in circumstances in which rapid information about mycobacterial species would alter the persons care or in a situation where a large contact-tracing initiative is being explored.[9] Other guidance advises rapid detection of MDRTB is also recommended on the basis that filtering face piece (FFP3) masks respiratory protection should be used until MDRTB has been

excluded.[10] The Public Health England (PHE) position statement published in July 2013 states that molecular testing of *M. tuberculosis* complex (MTBC) on respiratory samples is superior to smear microscopy for the diagnosis of TB and should be accessible in all areas of Scotland, England and Wales with results available within 1 - 2 working days of the sample being taken.[11]

The objective of this study was to evaluate the performance of Xpert MTB/RIF for detection of pulmonary TB in patients from the Tayside region of Scotland which has a low TB and HIV prevalence. In addition we aimed to examine the quantitative capabilities of Xpert MTB/RIF in relation to predicting auramine stain grading, as well as looking at TTP of culture.

Materials and Methods

Study Design and clinical samples

This is a retrospective review and analysis of data collected for clinical purposes. Respiratory samples (sputum, bronchoalveolar lavage (BAL), induced sputum, and endotracheal aspirates (ETA)) were submitted over a 3 year period (February 2011 to March 2014) and tested by Xpert MTB/RIF at the Department of Medical Microbiology, Ninewells Hospital and Medical School, Dundee (NHD). Samples came from both hospital inpatient and community settings and were sent for graded auramine smear microscopy and culture using solid Löwenstein-Jensen (LJ) media (containing pyruvate as a growth supplement) and BACTEC MGIT 960 liquid media at the Scottish Mycobacteria Reference Laboratory (SMRL) at the Royal Infirmary of Edinburgh.

Xpert MTB/RIF

At NHD, a minimum of 1 ml raw sputum or BAL was collected from samples. Xpert TB/RIF was performed on a GeneXpert instrument with GX2.1 software (GX) according to the manufacturer's instructions. A 2ml volume of sample reagent was added to each sample and shaken vigorously 10-20 times. This was incubated for 15 minutes at room temperature. At a point between 5-10 minutes of incubation the sample was shaken vigorously again 10-20 times. The liquefied sample was aspirated into a sterile transfer pipette until the meniscus was above the minimum mark then added to the Xpert MTB/RIF cartridge and then run on the machine according to the manufacturer's instructions. The GeneXpert DX System interpreted Xpert MTB/RIF results depending on the cycle threshold (Ct) value of MTB target present in the sample. When MTB was detected results were displayed as high (Ct <16), medium (Ct 16-22), low (Ct 22-28) or very low (Ct >28). These are known as the Xpert MTB/RIF assay load. Negative results were displayed as MTB not detected. Rifampicin resistance was reported as either detected, not detected or indeterminate.

Sample processing

Respiratory samples were sent to SMRL where they were liquefied and concentrated using Sputasol (1:1 v/v; Oxoid) and a loopful of sediment used to prepare a smear for auramine phenol microscopy using standard laboratory methods.[12] The number of AFB present was scored as: + (few AFB, one to 10 bacilli in 10 fields), ++ (moderate AFB, one to 10 bacilli per field) or +++ (many AFB, 10 or more bacilli per field). Specimen decontamination was performed with 2% NaOH-NALC and the pellet resuspended in 1.5ml phosphate buffer. 0.5ml was used to inoculate both a LJ with pyruvate slope and a BACTEC MGIT 960 tube (Becton Dickinson) and 0.5ml was stored at -20^{o}C for further additional molecular testing where appropriate.

Culture identification

Cultured mycobacteria were identified using GenoType® MTBC GenoType® Mycobacteria CM v1.0 or GenoType® Mycobacteria AS v1.0 (HAIN Lifescience) following the manufacturer's instructions.[13,14] MGIT and LJ cultures were considered to have a negative result if no mycobacterial growth was seen after 6 or 12 weeks of incubation respectively.

All MTBC-positive specimens were tested for resistance to RIF and INH using GenoType® MTBDR*plus* v1.0 and v2.0 (HAIN Lifescience) to confirm the Xpert RIF-resistance result and confirmed by phenotypic methods.[15,16]

Statistical analysis

Clinical and laboratory data were stored in Microsoft Excel analysed using simple descriptive methods in IBM SPSS version 23. Consecutive samples from the same patient were included in our analysis. The sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV) of Xpert MTB/RIF were calculated with 95% confidence intervals (CI).[17] Chi-squared test for trend was used to compare proportions across ordered categories. A Kruskal-Wallis test was used to compare the distributions of times to positivity between subgroups.

Ethics Approval

Caldicott guardian approval was gained from NHS Tayside in order to enable appropriate information sharing and protect the confidentiality of patients.

Results

Performance of Xpert MTB/RIF

A total of 2103 Xpert MTB/RIF tests were performed on samples from 1299 patients (Figure 1). The number of samples per patient ranged from 1 to 14, with a median of 1. A total of 38 samples were invalid on Xpert MTB/RIF testing (19 sputum, 15 BAL, 3 ETA and 1 induced sputum). A further 79 samples had insufficient material for further testing. Of the 1986 remaining samples, 35 were contaminated on culture, leaving 1951 samples from 1211 patients that received both Xpert MTB/RIF and culture (1141 sputa, 754 BAL, 51 ETA and 5 induced sputa).

In total 48 (2.5%) grew MTBC and 1903 were MTBC- negative using culture (97.5%). Of the 1903 MTBC-negative samples, 97 grew non-tuberculous mycobacteria (NTM) and no MTB was detected using Xpert MTB/RIF. If culture is used as the gold standard, the overall sensitivity of Xpert MTB/RIF was 95.8%, specificity 99.5%, PPV 82.1%, and NPV 99.9%. (Table 1)

Figure 1. Overview of Xpert MTB/RIF Tests and Cultures Performed

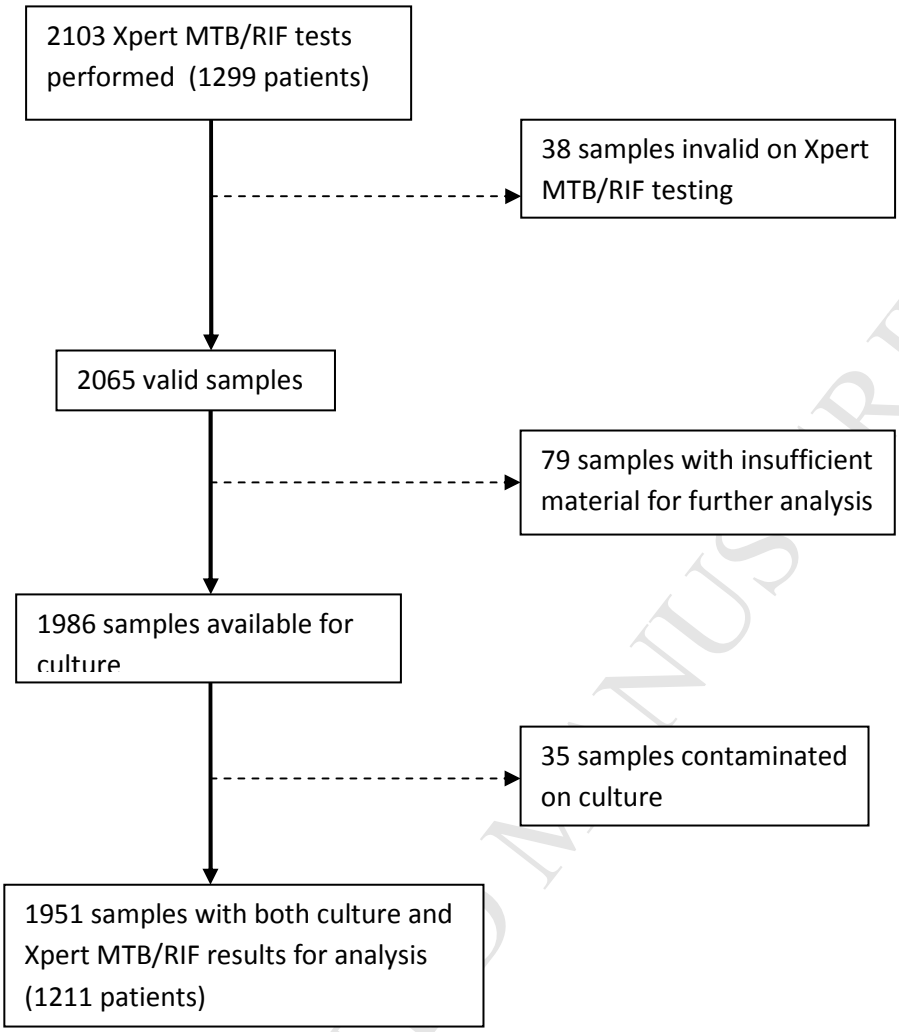


Table 1. Overall Performance Characteristics of Xpert MTB/RIF For All Respiratory Samples

Xpert MTB/RIF vs. Culture	Number detected/Total	Estimate %	95% CI
Sensitivity	46/48	95.8%	86.0, 98.8%
Specificity	1893/1903	99.5%	99.0, 99.7%
PPV	46/56	82.1%	70.2, 90.0%
NPV	1893/1895	99.9%	99.6, 100.0%

Of note, two false negative Xpert MTB/RIF results from two different patients were obtained. The first patient had four sputa and one BAL which were all AFB smear-negative with no MTB detected using Xpert MTB/RIF. All showed no mycobacterial growth except from a sputum that grew MTB at 21 days. Interestingly, lymph node tissue from this patient was AFB smear positive with MTB detected by in-house IS6110 PCR (Ct 25.1) and culture at the reference laboratory. The other patient had a sputum sample that was AFB smear negative with no MTB detected using Xpert MTB/RIF but grew MTB at 25 days. In addition two other sputa collected at this time were AFB smear negative but Xpert MTB/RIF positive then subsequently grew MTB. Approximately 3 months after the study period this patient had further sputa which were AFB smear-positive and grew MTB.

Two BAL and 8 sputum produced false-positive Xpert MTB/RIF results (6 with low level and 4 with very low levels of MTB detected) from 7 different patients. Of these, six patients had one false-positive Xpert MTB/RIF result and one patient had four false-positive Xpert MTB/RIF results. Five patients had MTB detected in subsequent sputum samples which grew MTB. For the remaining two patients, one had 3 sputa AFB smear- and culture-negative. One of the samples had a low reading (give Ct 26.9) using Xpert MTB/RIF, but no MTB detected from a subsequent sputum. The reference lab direct in-house IS*6110* PCR was MTBC-positive and clinically the chest radiograph and CT scan was suggestive of infection such as TB. This patient received a full course of TB therapy with a good response. The last patient had 4 sputum submitted which were AFB smear- and culture-negative and two citrated blood samples that did not grow mycobacteria. The Xpert MTB/RIF result for a BAL from this

patient was very low (give Ct 33.3). They responded to standard antibiotic treatment for pneumonia and following review in the infectious disease clinic after admission it was decided there was no good evidence the patient had TB. It appears therefore that this patient had a true false-positive Xpert MTB/RIF result.

For the 51 ETA and 5 induced sputum all cultures were MTBC negative. The sensitivity, specificity, NPV, PPV and 95% confidence interval (CI) of Xpert MTB/RIF for sputum and BAL samples is shown in Table 2.Within those with a culture result available, the PPV increased with the increasing category of the Xpert MTB/RIF assay load results.

Table 2. Sensitivity, Specificity, NPV, PPV of Xpert MTB/RIF for Sputum and BAL Samples

Xpert MTB/RIF vs. Culture	**Sputum numbers**	**Estimate %**	**95% CI**	**BAL numbers**	**Estimate %**	**95% CI**
Sensitivity	41/43	95.3%	84.5, 98.7%	5/5	100.0%	56.6, 100.0%
Specificity	1090/1098	99.3%	98.6, 99.6%	747/749	99.7%	99.0, 99.9%
PPV	41/49	83.7%	71.0, 92.7%	5/7	71.4%	35.9, 91.8%
NPV	1090/1092	99.8%	99.3, 99.9%	747/747	100.0%	99.5, 100.0%

Detection of Rifampicin Resistance

One patient in the study was diagnosed with multi-drug resistant (MDR) TB using direct GenoType MTBDRplus at SMRL. Although two wild-type rpoB bands were not detected, no mutation was identified using this assay suggesting that another rpoB mutation was present to confer resistance. Nine samples submitted from this patient were found to contain MTB by Xpert MTB/RIF and the presence of a rpoB mutation was indicated by reduced binding of Probe B during PCR resulting in a >3.5 Ct spread between the earliest and latest Ct values.

Xpert MTB/RIF and Microscopy

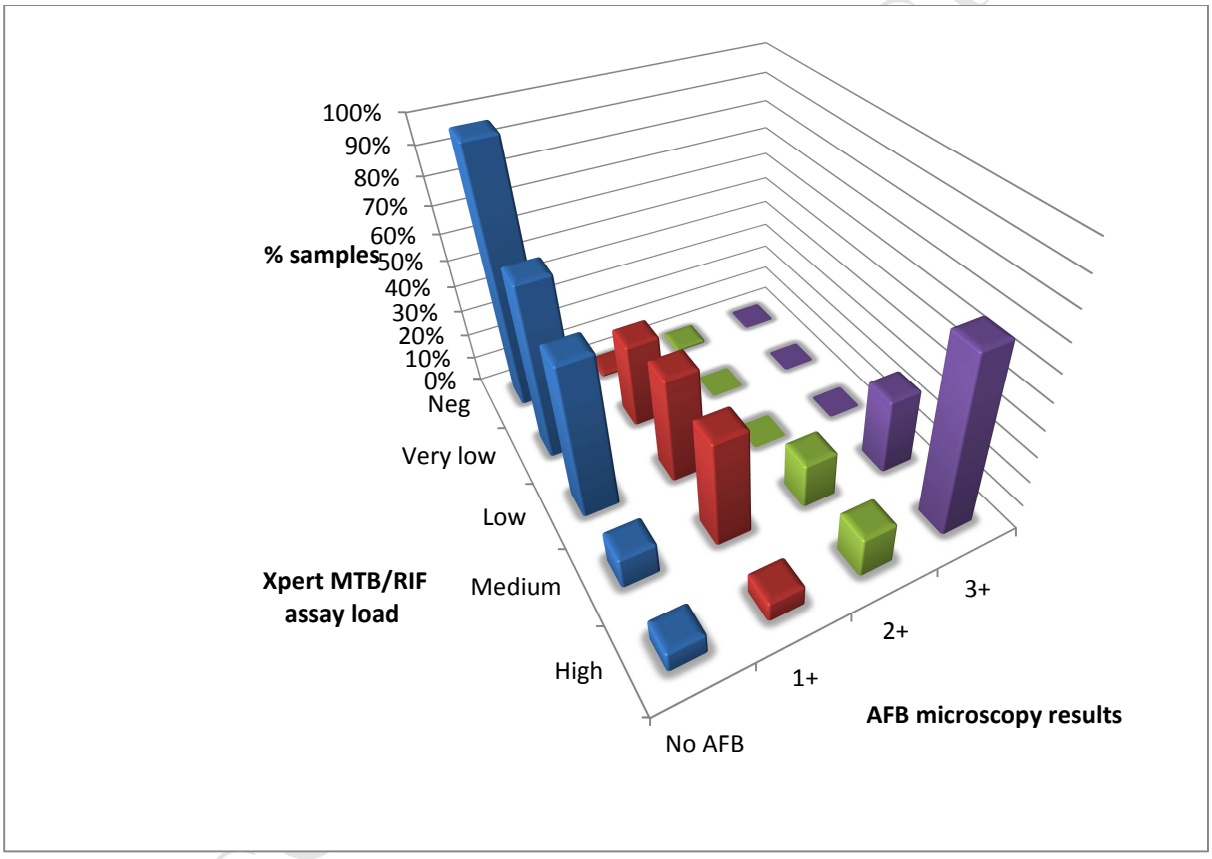
Overall there were 1927 samples in which microscopy was carried out. It can be seen in Table 3 and Figure 2 that there is a clear positive relationship between AFB microscopy grade and the Xpert MTB/RIF assay load results even when 97 atypical mycobacteria are included. Of these there were 57 non-tuberculous mycobacteria (NTM) with no AFB seen on microscopy, 20 with few AFB seen on microscopy, and 20 with moderate AFB seen on microscopy. The percentages in the upper two microscopy groups increased as the Xpert MTB/RIF assay load results increased, from 0%, 0% and 47.0%, to 84.6%. A chi-squared test for trend confirmed the statistical significance of these findings ($p<0.001$).

Table 3. Acid Fast Bacilli Microscopy and Xpert MTB/RIF Assay Load Results

Xpert MTB/RIF assay load results	Microscopy grouped	Negative (no AFB)	P+ (few AFB)	P++ (moderate AFB)	P+++ (many AFB)	Total
MTB not detected	**Count**	1825 (57 atypical)	23 (20 atypical)	20 (20 atypical)	3	1871
	Percentage	97.5	1.2	1.1	0.2	100.0
Very low	**Count**	6	3	0	0	9
	Percentage	66.7	33.3	0.0	0.0	100.0
Low	**Count**	10	7	0	0	17
	Percentage	58.8	41.2	0.0	0.0	100.0
Medium	**Count**	2	7	3	5	17
	Percentage	11.8	41.2	17.6	29.4	100.0
High	**Count**	1	1	2	9	13
	Percentage	7.7	7.7	15.4	69.2	100.0

Total Xpert MTB/RIF assay load results	**Count**	1844	41	25	17	1927
	Percentage	95.7	2.1	1.3	0.9	100.0

Figure 2. Relationship between Xpert MTB/RIF Assay Load and AFB Microscopy Results



Xpert MTB/RIF and TTP

We examined time to positivity (TTP) in the 48 culture positive samples (Table 4). This ranged from 6 to 56 days and was highly positively skewed. The median TTP was 10 days. There was a clear reduction in the median TTP as the Xpert MTB/RIF assay load results increased. A Kruskal-Wallis test comparing times to positivity in the Cepheid PCR categories showed a significant difference with p=0.005.

Table 4. Xpert MTB/RIF assay load results and TTP

Xpert MTB/RIF assay load results	Samples with MTB/RIF result available	Number of samples culture positive	Median TTP (days)	Percentile 25	Percentile 75
MTB NOT DETECTED	1974	2	23	21	25
VERY LOW	9	5	16	15	20
LOW	17	11	12	8	21
MEDIUM	17	17	10	9	14
HIGH	13	13	7	6	8

Conclusion

We believe this study reports the results of the largest number of Xpert MTB/RIF tests carried out in a low prevalence area to date. Taking culture as the gold standard, the overall sensitivity of Xpert MTB/RIF was 95.8%, specificity 99.5%, PPV 82.1%, and NPV 99.9%.

In this study, the Xpert MTB/RIF had a sensitivity and specificity of 95.3% and 99.3% respectively when used on sputum samples. These figures are similar to previously reported studies in high prevelence areas.[6] The sensitivity of Xpert MTB/RIF on BAL specimens was

found to be 100.0% which is higher in comparison to the findings of a previous study where is was found to be 81.6%.[18] A recent study involving the Xpert MTB/RIF in Canada found it had a sensitivity of 46% and specificity of 100% for detection of MTB from induced sputum samples. The authors concluded that paucibacillary disease and dilution of the sample in the process of sputum induction may have accounted for its low sensitivity.[19] There were small numbers of ETA and induced sputum submitted all of which were culture-negative. In particular the 5 induced sputa were sampled from 5 different patients. This could be said to have been observed as having 100% specificity but we cannot say if this would be true using a larger number of samples

The percentage of samples which were invalid using Xpert MTB/RIF was 1.8% (a total of 38 samples). Invalid results indicate that the sample processing control (SPC) has failed because the sample was either not properly processed or PCR was inhibited. In real life comparisons of methods there may be invalid results which can affect the usefulness of tests. Our rate of invalids was lower than previously reported.[19]

It has previously been described that the Xpert MTB/RIF is not specific for the detection of rifampicin resistance as silent mutations in the *rpoB* gene can give rise to the detection of false-positive rifampicin resistance.[20] We cannot comment on whether false positive readings can arise from silent mutations as we only found one patient who had MDRTB. Xpert MTB/RIF detected rifampicin resistance in 9/9 samples and this was confirmed by the reference laboratory's standard methods. Another limitation of this study is that we cannot provide the HIV status of the Xpert MTB/RIF tested patients.

We found a clear positive relationship between AFB microscopy grade and the Xpert MTB/RIF assay load results and this was statistically significant ($p<0.001$). Additionally, a clear reduction in the median TTP as the Xpert MTB/RIF assay load results increased was found. A Kruskal–Wallis test comparing distributions of times to positivity in the Xpert MTB/RIF categories showed a significant difference ($p=0.005$). These results support similar findings from previous studies.[21,22] Molecular testing performed directly on respiratory samples is likely to be appropriate for the assessment of infectivity and Xpert MTB /RIF has recently been approved for infection prevention and control use in the United States. A 2-specimen Xpert strategy was found to be most efficient in minimizing airborne infection isolation time while identifying all TB cases among individuals with presumptive TB.[23] We

believe we found only one true false positive Xpert MTB/RIF result from a patient who had consolidation on chest radiograph and adrenal lesions for 5 years however they responded to standard treatment for pneumonia. In low prevalence TB settings false positives should be expected. They can lead to significant clinical and public health implications as it did in this case in which a problem assessment group meeting was held with the local Health Protection Team.

Our results suggest that Xpert MTB/RIF could provide accurate results in low TB prevalence settings. In particular, this assay had a very good NPV which could be useful for ruling out a diagnosis of pulmonary TB. Serious consideration should be given to using Xpert MTB/RIF as a replacement for microscopy in low prevalence situations. Delays in transport of samples to reference laboratories can detrimentally affect culture results. Introducing molecular tests such as Xpert MTB/RIF potentially leads to production of rapid results which would enable earlier detection or exclusion of pulmonary TB and can be used to influence treatment decisions and appropriate infection prevention and control measures. Molecular testing for detection of MTBC performed directly on respiratory samples has the potential to be superior to smear microscopy for the diagnosis of TB, although culture should remain the gold standard; essential for subsequent drug susceptibility testing and MTBC genotyping.

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Potential conflicts of interest

Conflicts of interest: none

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Highlights

- Xpert MTB/RIF gave accurate results in a low TB and HIV prevalence setting
- It had a very good NPV useful for ruling out pulmonary TB
- Auramine microscopy grade and Xpert MTB/RIF assay load correlated
- Median TTP reduced as Xpert MTB/RIF assay load increased
- Molecular testing respiratory samples may be appropriate for infectivity assessment