The performance of the Xpert MTB/RIF Version G4 in a low tuberculosis incidence setting

Sir,

Mycobacterial testing continues to evolve with improving technology, affecting the way we diagnose, report and interpret mycobacterial results. Prior to introduction of the GeneXpert Ultra, our Mycobacterial Reference Laboratory (MRL) at the Victorian Infectious Diseases Reference Laboratory (VIDRL) in Melbourne, utilised the Xpert MTB/RIF assay, Version G4 (Cepheid, USA), to detect *Mycobacterium tuberculosis* complex (MTBC) and potential resistance to rifampicin via mutations within a region of the *rpoB* gene.¹

Prior literature on the performance of this Xpert MTB/RIF assay relates to previous versions of the assay.¹ Here, we sought to characterise the performance of the G4 Xpert MTB/RIF for detection of MTBC and identification of potential rifampicin resistance in a low TB prevalence population.

Retrospective data at the state MRL were analysed from 21 June 2012 to 14 June 2018. Processing of primary specimens for acid fast bacilli (AFB) staining, predominantly by the Ziehl–Neelsen method, and mycobacterial culture were routinely performed at the referring laboratory. All mycobacterial culture positive samples were requested to be referred for further characterisation.

Respiratory samples were processed in line with recommendations.^{2,3} Fluids and homogenised extra-pulmonary tissue samples were processed using an accredited in-house method. For paraffin embedded tissue, samples were dewaxed and lysed with a Tris-HCl digestion buffer prior to testing. The 543 bp region of the *rpoB* gene was sequenced using the primers and methods described by Campbell *et al.*⁴ on isolates that had an *rpoB* mutation detected via Xpert MTB/RIF assay. Sequences were aligned with the H₃₇Rv wild-type sequence to assess for variance.

Phenotypic susceptibility testing was performed on MTBC isolates grown from the same or contemporaneous patient samples using the BD BACTEC MGIT 960 module (Becton Dickinson Microbiology Systems, USA). Phenotypic rifampicin susceptibility was tested at a critical concentration of 1 µg/mL.⁵ Isolates found to have a single nucleotide polymorphism (SNP) that were not known to be associated with rifampicin phenotypic resistance were re-tested using lower concentrations of rifampicin (0.125 µg/mL, 0.25 µg/mL, 0.5 µg/mL) to determine if low level phenotypic resistance was present.

Data from all Xpert MTB/RIF tests performed at the MRL were extracted, including AFB results, culture results and, where performed, MTBC rifampicin phenotypic susceptibility and *rpoB* gene sequencing. Additional data included patient demographics and clinical details such as previous treatment and susceptibility results. Multiple samples from individual patients were included.

Over the study period, 1849 Xpert MTB/RIF tests were performed, of which 681 samples (36.8%) were Xpert MTB/RIF positive (Fig. 1A). This rate decreased to 29.2% when only primary clinical samples were included. Of the primary samples, 48.7% were non-respiratory.

A corresponding positive MTBC culture was identified for 47.3% (460/972) primary samples tested by Xpert MTB/RIF (Table 1). Of the 460 MTBC culture positive primary

samples, 85% (390/460) were positive. In addition, one sample positive by Xpert MTB/RIF and confirmed by second assay⁶ was MTBC culture negative, attributed to overgrowth by *Mycobacterium avium* complex. A further 4.2% (18/430) demonstrated discordant Xpert MTB/RIF assay positive, culture negative results. However, 12 of these 18 positive samples were from patients who either had a history of prior or current treatment. The overall sensitivity was 84.8% [390/460, 95% confidence interval (CI) 81.2–87.9%] and specificity 96.3% (19/493, 95% CI 94.3–97.8%). The Xpert MTB/RIF had a high negative predictive value of 87.6% (95% CI 85.0–89.7%) when compared to culture.

The Xpert MTB/RIF performance was assessed on AFB positive and negative samples. AFB results were unavailable for 15 samples (Table 2A). The positive predictive values for both AFB positive and AFB negative samples were high, 99.4% (95% CI 96.1–100%) and 98.6% (95% CI 95.7–99.5%) respectively (Table 2B). This reflects a combination of a highly specific assay, coupled with low rates of re-testing of current or previously treated patients. Similarly, the negative predictive value for AFB positive samples was high, 97.5% (95% CI 84.7–99.6%), representing a higher potential burden of target. In samples which were AFB negative, the negative predictive value decreased to 86.3% (95% CI 83.6–88.6%) but remained high. AFB negative samples from a non-respiratory source had a lower detection rate than respiratory samples (70.7% vs 84.3%), likely representing a relatively low bacterial sample load, less efficient DNA extraction or a combination of both.⁷

Other authors have previously described Xpert MTB/RIF assay positive, MTBC culture negative results as false positive results, attributed to the assay's suboptimal specificity.⁸ The Xpert MTB/RIF is designed to detect DNA, not viability. Treated patients may not have cleared the non-viable MTB at the time of testing.⁸ This factor needs to be considered when interpreting positive results in this cohort. This issue is expected to be magnified with the introduction of the more sensitive Xpert MTB/RIF Ultra⁸ which utilises multi-copy targets, in addition to the *rpoB* gene, to improve the limit of detection.

A further benefit of the Xpert MTB/RIF assay is the ability to detect potential rifampicin resistance. In this study, the positive and negative predictive values for resistance detection were 93.6% (95% CI 78.5–98.3%) and 100%. A resistance mutation was detected in 4.6% of samples (31/681 positive results, from 29 unique patients). There was insufficient signal to determine presence of a mutation in six samples; two were phenotypically rifampicin susceptible, and four had no corresponding culture. There was no Xpert MTB/RIF undetected phenotypic resistant isolates. Given the numbers in our study, this is not surprising. The Xpert MTB/RIF assay targets the 81 bp region of the *rpoB* gene where 95–97% of resistance-conferring mutations are located.⁹ The Ser531Leu amino acid change was the most frequently observed SNP, seen in 16 of our samples, consistent with published reports¹⁰ (see Fig. 1B). Other SNP changes detected that displayed phenotypic resistance and reduced rifampicin susceptibility have also been described in other studies.¹⁰ One sample was found to have Leu533Pro, a SNP described as conferring low level rifampicin resistance,¹¹ that tested phenotypically resistant in our laboratory; however, this would not have been reliably detected on previous versions of the assay. The clinical significance of low level rifampicin resistance is unresolved; however, patients

A

B

Fig. 1 (A) Xpert MTB/RIF result by sample type tested from samples received between 21 June 2012 and 14 June 2018 at the Mycobacterial Reference Laboratory. (B) Phenotypic susceptibility mapped against the *rpoB* sequencing results of Xpert MTB/RIF positive patients with amino acid substitutions detected when aligned with the H₃₇Rv wild-type sequence. R, resistance; RS, reduced susceptibility; S, substitution but susceptible.

infected with such isolates often fail treatment or relapse.¹⁰ Importantly, the MGIT broth susceptibility testing doesn't reliably detect low level phenotypic resistance.¹⁰ The Xpert MTB/RIF permits identification of these isolates and may allow altered management, including closer monitoring of these at risk patients.

Two Xpert MTB/RIF positive resistance results were phenotypically susceptible. One result was subsequently

identified as a true false positive resistance detection. This reflects a potential issue with either the sample preparation, test process or result interpretation on initial testing. The second result had a SNP detected with potential resistance association but tested phenotypically susceptible. Of note, the same mutation was detected in a different isolate that was phenotypically resistant to rifampicin. This highlights that the significance of many SNP changes have yet to be fully elucidated within

Table 1 Xpert MTB/RIF concordance with culture result

Culture result	Positive/Number tested (% positive)
No culture	51/603 (8.5%)
Culture negative	18 ^a /430 (4.2%)
Non-tuberculous mycobacteria/Nocardia	1 ^b /82 (1.2%)
MTBC	390/460 (84.6%)

^a 12/18 noted to have a prior history of treatment or treated at time of collection.

^b One sample overgrown with *Mycobacterium avium* complex.

Table 2 (A) Xpert MTB/RIF result by MTBC culture positive separated by AFB stain result and sample type; (B) Comparison of MTBC culture and Xpert MTB/RIF results by AFB stain result of samples received at the MRL

(A) Xpert MTB/RIF result by positive MTBC culture				
Sample type	AFB positive		AFB negative	
	Positive/Number tested (% positive)		Positive/Number tested (% positive)	
Respiratory	137/138 (99.3%)		86/102 (84.3%)	
Cerebrospinal fluid (CSF)	1/1 (100%)		4/6 (66.7%)	
Extra-pulmonary tissue/fluid	27/27 (100%)		82/108 (75.9%)	
Intrathoracic tissue/fluid sample	7/7 (100%)		30/50 (60.0%)	
Paraffin embedded tissue	0		5/6 (83.3%)	
All samples	172/173 (99.4%)		207/272 (76.1%)	

(B) Comparison of MTBC culture and Xpert MTB/RIF by AFB stain result				
	AFB positive		AFB negative	
	Culture positive	Culture negative	Culture positive	Culture negative
Xpert positive	172	1	207	3
Xpert negative	1	39	65	410

MTBC, and that the Xpert MTB/RIF should be used as a guide to determine potential presence of resistance.

In this retrospective data analysis, the Xpert MTB/RIF assay demonstrated high positive and negative predictive values in both AFB positive and negative samples. Since conducting this analysis, the GeneXpert Ultra has been introduced which includes multi-copy targets, further improving the limit of MTBC detection. The impact on specificity of this change needs to be established. The Xpert MTB/RIF assay has demonstrated its reliability and robustness as the cornerstone of rapid MTBC diagnostics. This report provides a clear benchmark with which to compare newer generation assays.

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***Ignavigranum ruoffiae* associated with skin abscess**

Sir,

We describe the isolation of *Ignavigranum ruoffiae* from a skin abscess in a patient attending our emergency department. A 62-year-old female patient presented to hospital with an infected sebaceous cyst and overlying cellulitis. She underwent operative debridement with tissue/sebaceous cyst collected for histopathology and pus sent for microbiological analysis. Gram-positive cocci were seen on microscopy and the patient was discharged on oral flucloxacillin with good clinical response. Pure growth of an unusual pathogen,