Interpreting very low *Mycobacterium tuberculosis* detected on Xpert *Mycobacterium tuberculosis*/rifampicin

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ABSTRACT

The development and rollout of the Xpert® *Mycobacterium tuberculosis*/rifampicin assay for the GeneXpert platform is considered an important breakthrough in the fight against tuberculosis. Xpert though robust is known to have issues that occur with very low load of tuberculosis detection, wherein it is recommended to confirm resistance if resistance is not suspected using another genotypic test.

KEY WORDS: Cartridge-based nucleic acid amplification test, pyrosequencing, *rpoB*, tuberculosis, Xpert *Mycobacterium tuberculosis*/rifampin

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INTRODUCTION

The development of the Xpert® *Mycobacterium tuberculosis*/rifampicin (MTB/RIF) assay for the GeneXpert platform was completed in 2009 and is considered an important breakthrough in the fight against tuberculosis (TB). For the first time, a molecular test is simple and robust enough to be introduced and used outside conventional laboratory settings. MTB is detected by the five overlapping molecular probes (Probes A–E) that collectively are complementary to the entire 81 bp *rpoB* core region.[1-4] MTB is identified when at least two of the five probes give positive signals with a cycle threshold (Ct) of ≤38 cycles and that difference is by no more than a prespecified number of cycles. The *Bacillus globigii* internal control is positive when the single *B. globigii*-specific probe produces a Ct of ≤38 cycles. The standard user interface indicates the presence or absence of MTB and the presence or absence of RIF resistance and a semiquantitative estimate of the concentration of *Bacilli* as defined by the Ct range (high, 28). Assays that are negative for MTB and for the *B. globigii* internal control are reported as invalid assays. When performed on unprocessed sputum samples, the assay can generate results within 2 h with <15 min of hands-on time. The basis for the detection of RIF resistance is the difference between the first (early Ct) and the last (late Ct) MTB-specific beacon (ΔCt). The system was originally configured such that resistance was reported when ΔCt was >3.5 cycles and sensitive if ≤3.5 cycles. Since the assay terminates after 38 cycles, the assay was deemed indeterminate for RIF resistance if the first probe Ct is >34.5 cycles and the last probe has a Ct of >38 cycles.[4] From May 2010, the automated detection of RIF resistance was modified using a new ΔCt cutoff in order to improve the specificity for RIF resistance detection.[5]

MATERIALS AND METHODS

At our tertiary care hospital in Mumbai, we observe a lot of resistance with a bias toward nonresponders. We assessed Xpert data from June 2017 to May 2018, where of the total 10,809 number of Xpert MTB/RIF processed, 5990 were MTB

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not detected and the remaining 4819 were MTB detected. Among these 4819 MTB-positive samples, 166 samples were detected with very low load of MTB. Table 1 depicts the number of samples and the load that were diagnosed by Xpert.

RESULTS

A total of 166 samples were found to be Xpert very low for the detection of MTB, of these 40 were Xpert RIF resistant and 96 were Xpert RIF susceptible. Among the RIF susceptible, mycobacterial growth indicator tube (MGIT) drug susceptibility testing (DST) RIF was susceptible in 92 and resistant in 4. These four samples have been sent for the whole-genome sequencing, and the results are awaited.

Among the Xpert RIF resistant, MGIT DST was resistant in 27 samples and susceptible in 13. Pyrosequencing (PSQ) showed mutation in 27 samples that were DST RIF resistant. Thirteen samples were susceptible to RIF by MGIT DST; among these, three samples showed mutation with PSQ. These mutations fall under the disputed category, for which the gold standard is challenged [Figure 1]. There were ten samples that were susceptible by both PSQ and MGIT DST, and Xpert was resistant due to Ct difference.

Analysis of 40 samples where Xpert MTB very low with RIF resistance was done. The PSQ performed for all these 40 samples was compared with the results of MGIT RIF DST [Table 2]. There were ten samples that showed a discrepancy between Xpert results and MGIT DST. The PSQ results of these samples showed no mutation in the RIF resistance determining the region. When the Xpert MTB/RIF Ct was evaluated, 8/10 samples showed a Ct difference of >3.5, whereas two samples showed probe Ct value to be zero. It was observed that when any of the Ct values were absolute zero, a mutation was observed in PSQ and the corresponding DST was also resistant to RIF. This was observed in 30/40 samples. There was a single sample that showed Ct difference among the probes, but it showed a mutation in PSQ and was found to be RIF resistant with MGIT DST. There were three samples that showed mutation in PSQ, but the DST RIF was susceptible as these mutation come under the disputed mutation category, wherein the MGIT DST at the critical concentration is sensitive.

Table 1: The segregation of the *Mycobacterium tuberculosis* complex positive as per the load of *Mycobacterium tuberculosis* complex detection in Xpert

MTB-detected load	*n*	RIF status	
		Susceptible	Resistant
High	2574	1846	728
Medium	847	580	267
Low	1232	940	292
Very low	166	126	40

MTB: *Mycobacterium tuberculosis* complex, RIF: Rifampicin

DISCUSSION

Currently, the primary tool for the diagnosis of TB is a cartridge-based nucleic acid amplification test. Xpert MTB/RIF test. It features in the diagnostic algorithm in many countries. Studies have shown that Xpert has good sensitivity and specificity of MTB and RIF resistance and is reported to be a game changer for the detection of TB.[2]

Our data have shown that there is a possibility that false RIF resistant results on 10/40 samples. All these samples showed a delayed Ct for MTB positive and positive analyte for all the probes present. Contrary to this, when there is a complete dropout of any probe, the result is correlated with PSQ and MGIT RIF DST. This was observed among 27/40 samples. This is in concordance with previous studies in the literature.[6,7] Single sample with delayed Ct for all probes showed a mutation in PSQ and subsequent resistance to RIF in MGIT DST.

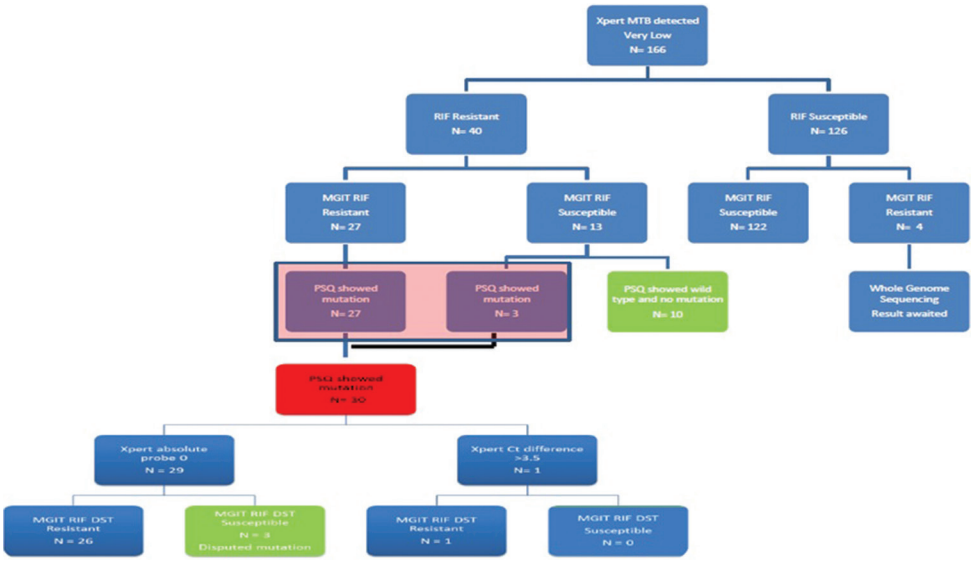

Figure 1: Flow chart depicting the segregation of Xpert *Mycobacterium tuberculosis* detected very low

Table 2: Cycle threshold values of the 40 samples that were Xpert resistant very low, pyrosequencing, and Mycobacterial Growth Indicator Tube drug susceptibility testing results

Sample	Xpert – Ct of probes					PSQ RIF mutations	DST RIF
	A	B	C	D	E		
Biopsy	29.7	29.4	29.8	30.3	0	531 TTG	R
LN BX	28.6	28.7	28.6	29.3	0	531 TTG	R
Sputum	29	29.9	28.9	30	0	531 TTG	R
LN	29.9	28.8	30.1	30	0	531 TTG	R
Sputum	29.7	30	29.9	30.5	0	531 TTG	R
BX	29.5	34.9	29.4	30.1	31.4	WT	S
Bal	29.8	30.1	30	30.6	0	531 TTG	R
BX	30.5	31	31	31.3	0	531 TTG	R
Abscess	32	32.7	30.3	33.8	34.9	WT	S
Aspirate	28.3	30.3	28	28.9	32.6	WT	S
LN BX	33.4	32.2	32.7	33.4	0	531 TTG	R
Tissue	32.4	33	31.8	32.4	0	531 TTG	R
LN BX	32.1	31.8	0	32.4	34	522 TTG	S
Pus	29.1	0	30.8	31.3	32.4	516 GGC	R
Aspirate	37.8	37.4	36.3	40.9	0	531 TTG	R
Left elbow pus	29	29.1	29.4	29.7	0	531 TTG	R
Tissue	32.8	31.5	31.3	34.4	0	531 TTG	R
Sputum	30.2	30	30.2	30.9	0	531 TTG	R
Sputum	28	33	28	29.3	30	516 GTC	R
Tissue	32.2	31.8	31.6	33.3	0	531 TGG	R
CSF	0	31.2	0	0	31.1	WT	S
Biopsy	38.3	38	37.7	0	0	WT	S
Pus	28.1	0	29.1	30.3	29.6	516 GTC	R
Pus	29.2	30.2	28.4	30.3	0	531 TTG	R
LN	30.9	31.1	31.2	31.8	0	531 TTG	R
LN biopsy	29.3	30.1	29.9	30.6	0	531 TTG	R
Urine	28.3	30.3	28	28.9	0	531 TTG	R
Ct-guided BX	29	29.9	28.9	30	0	531 TTG	R
LN BX	33.6	32	32	35.4	0	531 TTG	R
Pericardial fluid	35.8	36.7	35.5	36.8	38.5	WT	S
Pleural tap	28.7	28.2	28.5	29.2	0	531 TTG	R
Tissue	24.4	25.3	24.9	25.6	0	531 TTG	R
Tissue	32.8	31.5	30.6	35	35.2	WT	S
Abscess	30.2	30.2	30.6	0	32.2	526 AAC	S
Biopsy	34.6	30.2	29.5	31.7	32.7	WT	S
Mesenteric LN	33.2	32	31.3	35.4	35.3	WT	S
Biopsy	28.9	0	29.3	31.3	32.2	516 TAC	S
Biopsy	33.2	31.7	31.6	35.1	35.9	WT	S
Tissue	28.4	30.6	31	31.8	0	531 TTG	R
Biopsy	29.6	31.4	29.8	32.3	0	531 TTG	R

LN: Lymph node, BX: Biopsy, WT: Wild type, S: Susceptible, R: Resistant, DST: Drug susceptibility testing, PSQ: Pyrosequencing, CSF: Cerebral spinal fluid, Ct: Cycle threshold, RIF: Rifampicin

WHO has recommended the use of Xpert MTB RIF for the testing of extrapulmonary samples,[8] HIV-positive cases, and pediatric cases. These samples are very often paucibacillary, and Xpert may diagnose with a delayed Ct and resistance to RIF. In cases, especially if RIF resistance is not suspected but resistance is detected, and there is a Ct difference, we suggest the use of another genotypic test followed by MGIT RIF DST be performed before the initiation of multidrug-resistant treatment.

CONCLUSION

The advent of Xpert Ultra with targets IS6110 and IS1081 for MTB may resolve this issue as it does not flag very low but gives the result as MTB-detected trace.[5] RIF resistance detection has also been improved in ultra by relying on the interpretation of the melting curves in the active site of *rpoB*.[9]

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Conflicts of interest
There are no conflicts of interest.

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