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Xpert ultra can unambiguously identify specific rifampin resistance-conferring mutations

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- 1 Xpert Ultra can unambiguously identify specific rifampicin resistance-conferring
- 2 mutations
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26	The deluge of data produced by XpertMTB/RIF (Cepheid) can help improve global
27	rifampicin-resistant tuberculosis (RR-TB) control strategies through molecular
28	epidemiological surveillance (1, 2). Recently, a new version of the test – Xpert Ultra
29	(hereinafter called Ultra) was released (3). Determining the relationship between RR-
30	conferring <i>rpoB</i> mutations, Ultra probes, and melting temperature shifts (Δ Tm) – the
31	difference between mutant and wildtype melting temperatures – allows Ultra results to
32	be utilized for rapid detection of RR-TB strains and related underlying <i>rpoB</i> mutations.
33	Methods
34	To determine the reliability of Ultra results for predicting specific mutations, we tested 10
35	rifampicin susceptible (RS)-TB, and 107 RR-TB strains harboring 36 unique RR-
36	conferring mutations, from the Belgian Coordinated Collections of Microorganisms in the
37	Institute of Tropical Medicine Antwerp, following a protocol previously described (2)
38	(Supplemental file 1). We then compared Ultra raw results with available rpoB
39	sequences of the strains.
40	Results
41	Overall, 29/30 (97%) mutations inside the Rifampicin Resistance Determining Region
42	(RRDR) were correctly identified by Ultra. Of concern, mutation His445Arg gave a "RIF
43	Resistance INDERTERMINATE" result among 3/4 strains tested while it was reported as
44	RR in the initial validation study (3). The silent mutation Thr444Thr was not reported as
45	RR (Figure 1). The RR-conferring mutations on codons 170, 250, 299, 482, and 491
46	situated outside the RRDR were not detected.
47	The probe reactions observed were largely in agreement with previous results (3) albeit
48	we noted that mutations Met434Val, Met434Thr and those in codon 435 were captured

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49 only by probe rpoB2; Ser450Leu and Ser450Trp were captured by both probes rpoB3 50 and rpoB4a, His445Arg was captured only by probe rpoB3; and Lys446Gln was 51 captured only by probe rpoB4.

52 All mutations except those in codon 450 were associated with a negative Δ Tm (Figure 53 2). The combination of Δ Tm values with the capturing probes enabled to differentiate 54 mutations in codons 428, 430, 431, 432, 434, 435, 441, 445, 446, and 452, including 55 disputed mutations (4) (Table 1). Mutation Asp435Tyr was unambiguously distinguished 56 from Asp435Val through probe rpoB2|△Tm|, while mutations Ser441GIn and Ser441Leu 57 were discriminated from the rest by $|\Delta Tm|$ values of probes rpoB2 and rpoB3. Mutations 58 His445Asp and His445Tyr were distinguished from disputed mutations His445Leu and 59 His445Asn through probe rpoB3 |∆Tm|. Ser450Leu was distinguished from Ser450Trp 60 by probe rpoB4A |\DeltaTm|. The indeterminate result associated with His445Arg may be 61 caused by its |\Delta Tm|=1.8°C compared with |\Delta Tm| typically exceeding 2°C for other mutations. Our recent experience with Ultra on diagnostic sputum samples only 62 63 pertained to the Ser450Leu and His445Asp mutations, for which the Δ Tm corresponded 64 exactly with the Δ Tm we observed on bacterial thermolysates. This should be validated more extensively, which is beyond the scope of our present study. 65

66 Conclusions

67 Our findings confirm the ability of Ultra to unambiguously identify a wide range of RRDR 68 mutations. With the unprecedented roll-out of XpertMTB/RIF and associated connectivity 69 solutions, such as DataToCare (Savics, Belgium) and GXAlert (SystemOne, USA) (2), 70 Ultra results may allow to rule-out transmission between RR-TB patients in a specific 71 setting (Figure S1), distinguish relapse from reinfection (5) (Figure S2), and resolve 72 discordance between an RR Ultra result and a low-level RS phenotypic result due to a

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- 73 disputed mutation. For such applications, it is key that Δ Tm values are included in the
- 74 exported results.
- 75 Acknowledgement
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101	Figure 1. Overview of Xpert Ultra test results. The observed probe reactions for each
102	RRDR mutation were overlaid on claimed probe coverage (light gray). Shown in black
103	are probe reactions concordant with manufacturer claims; in blue are probe reactions
104	missed by one probe but captured by another probe; and in red is a probe reaction
105	representing "RIF Resistance INDETERMINATE" result from 3 out of 4 strains tested.
106	Results in striped pattern were superimposed for greater visibility.
107	
108	Figure 2. Melting temperature shift (ΔTm) observed upon detection of a rifampicin
109	resistance (RR)-conferring rpoB mutation in the rifampicin resistance determining region
110	(RRDR) by Xpert Ultra. The y-axis reflects the melting temperature difference between
111	mutant and wildtype probe-amplicon hybrids (ΔTm), while the x-axis shows the
112	mutations we tested. The data points on the graph are ΔTm values grouped by
113	associated Ultra probe (differentiated by color) corresponding with a specific rpoB
114	mutation. X-axis labels in brown are disputed mutations.

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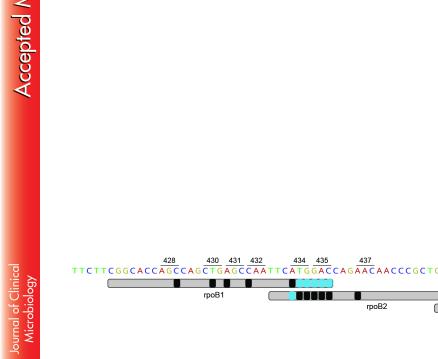
Table 1. Xpert Ultra raw results – capturing probe, wildtype melt peak temperature (Tm) range and mean, mutant (mut) Tm range, and absolute value of melting temperature shift (Δ Tm, range for multiple strains tested) – associated with rifampicin resistance-conferring mutations and corresponding nucleotide changes as determined by *rpoB* sequencing. Unique combiNDtions of Ultra probe and Δ Tm uNDmbiguously differentiate most but not all mutations within the Rifampicin Resistance Determining Region (RRDR), including disputed ones in italics.

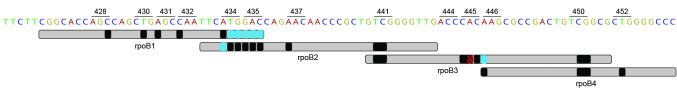
Mutation	Number of strains tested	Nucleotide change	Xpert Ultra Probe	wildtypeTm range mean	mutTm range	∆Tm range
Val170Phe	3	gtc > Ttc	ND	ND	ND	ND
Glu250Gly	2	gag > gGg	ND	ND	ND	ND
Arg299Cys	1	cgc > Tgc	ND	ND	ND	ND
Ser428Arg	1	agc > agG	rpoB1		65.8	3.5
*Leu430Pro	8	ctg > cCg	rpoB1	69.1-69.5 69.3	63.0-63.4	5.9 - 6.3
*Ser431Gly	1	agc > Ggc	rpoB1		66.4	2.9
*Gln432Glu	1	caa > Gaa	rpoB1		65.9	3.4
*Met434lle	1	atg > atA	rpoB2		69.8	3.2
*Met434Val	1	atg > Gtg	rpoB2		70.5	2.5
Me434Thr	1	atg > aCg	rpoB2		69.7	3.3
Asp435 Gly	1	gac > gGc	rpoB2	72.8-73.2 73	69.7	3.3
*Asp435Glu	1	gac > gaA	rpoB2		70.2	2.8
*Asp435Phe	1	gac > TTc	rpoB2		67.7	5.3
*Asp435Tyr	11	gac > Tac	rpoB2		68.6-68.9	4.0-4.4
*Asp435Val	5	gac > gTc	rpoB2		69.3-69.5	3.5-3.7
*Ser441GIn	1	tcg > CAg	rpoB2; rpoB3	72.8-73.2; 73;	68.3; 73.5	4.7; 2.3
*Ser441Leu	1	tcg > tTg	rpoB2; rpoB3	75.5-76.0; 75.75	70.0; 73.5	3; 2.3

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Thr444Thr	1	acc > acG	ND	ND	ND	ND
*His445Gln	1	cac > caG	rpoB3		72.2	3.6
*His445Gln	1	cac > caA	rpoB3		71.7	4.1
His445Gly	1	cac > GGc	rpoB3		70.9	4.9
His445Thr	1	cac > ACc	rpoB3	75.5-76.0 75.75	70.9	4.9
*His445Ser	1	cac > AGc	rpoB3		71.1	4.7
*His445Asp	3	cac > Gac	rpoB3		71.9-72.1	3.7-3.9
*His445Leu	2	cac > cTc	rpoB3		72.2-72.3	3.5-3.6
*His445Asn	2	cac > Aac	rpoB3		72.3-72.4	3.4-3.5
*His445Tyr	4	cac > Tac	rpoB3		72.5-72.6	3.2-3.3
*His445Arg	4	cac > cGc	rpoB3		73.9	1.9
*Lys446Gln	1	aag > Cag	rpoB4B	67.0-67.6 67.3	62.3	5.0
Ser450Phe	1	tcg > tTC	rpoB3	75.5-76.0 75.75	71.8	4.0
*Ser450Leu	14	tcg > tTg	rpoB3; rpoB4A	75.5-76.0	72.9-73.3; 73.3- 73.8	2.5-2.9; 3.6-6.5
*Ser450Trp	3	tcg > tGg	rpoB3; rpoB4A	75.75; 67.0-67.6 67.3	73.1-73.5; 70.6- 71.0	2.3-2.7; 3.3-3.7
*Leu452Pro	12	ctg > cCg	rpoB4B	67.0-67.6 67.3	61.2-61.6	5.7-6.1
Thr482Asn	1	acc > aAc	ND	ND	ND	ND
lle491Phe	10	atc > Ttc	ND	ND	ND	ND
lle491Val	1	atc > Gtc	ND	ND	ND	ND

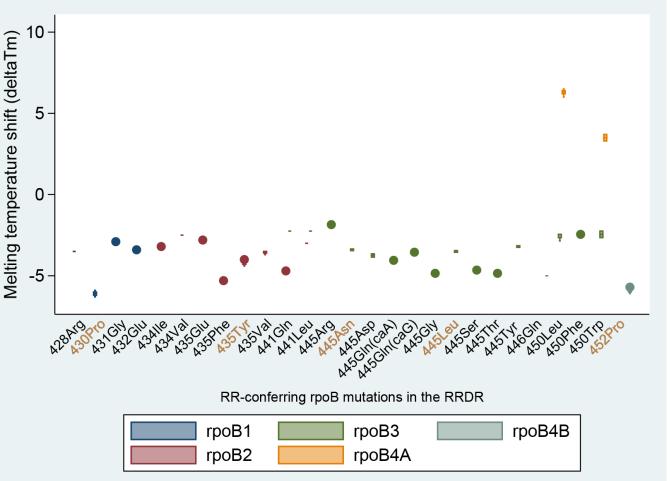
* RRDR mutations distinguishable by unique combinations of Ultra probes and ∆Tm; ND - strains that harbor corresponding mutations outside the RRDR yielded 'RIF Resistance NOT DETECTED' result





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