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Xpert Ultra can unambiguously identify specific rifampicin resistance-conferring mutations

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Running Head: Discriminatory Xpert Ultra data

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Introduction

The deluge of data produced by XpertMTB/RIF (Cepheid) can help improve global rifampicin-resistant tuberculosis (RR-TB) control strategies through molecular epidemiological surveillance (1, 2). Recently, a new version of the test – Xpert Ultra (hereinafter called Ultra) was released (3). Determining the relationship between RR-conferring *rpoB* mutations, Ultra probes, and melting temperature shifts (ΔTm) – the difference between mutant and wildtype melting temperatures – allows Ultra results to be utilized for rapid detection of RR-TB strains and related underlying *rpoB* mutations.

Methods

To determine the reliability of Ultra results for predicting specific mutations, we tested 10 rifampicin susceptible (RS)-TB, and 107 RR-TB strains harboring 36 unique RR-conferring mutations, from the Belgian Coordinated Collections of Microorganisms in the Institute of Tropical Medicine Antwerp, following a protocol previously described (2) (Supplemental file 1). We then compared Ultra raw results with available *rpoB* sequences of the strains.

Results

Overall, 29/30 (97%) mutations inside the Rifampicin Resistance Determining Region (RRDR) were correctly identified by Ultra. Of concern, mutation His445Arg gave a “RIF Resistance INDERTERMINATE” result among 3/4 strains tested while it was reported as RR in the initial validation study (3). The silent mutation Thr444Thr was not reported as RR (Figure 1). The RR-conferring mutations on codons 170, 250, 299, 482, and 491 situated outside the RRDR were not detected.

The probe reactions observed were largely in agreement with previous results (3) albeit we noted that mutations Met434Val, Met434Thr and those in codon 435 were captured

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

All mutations except those in codon 450 were associated with a negative ΔTm (Figure 2). The combination of ΔTm values with the capturing probes enabled to differentiate mutations in codons 428, 430, 431, 432, 434, 435, 441, 445, 446, and 452, including disputed mutations (4) (Table 1). Mutation Asp435Tyr was unambiguously distinguished from Asp435Val through probe rpoB2|ΔTm|, while mutations Ser441Gln and Ser441Leu were discriminated from the rest by |ΔTm| values of probes rpoB2 and rpoB3. Mutations His445Asp and His445Tyr were distinguished from disputed mutations His445Leu and His445Asn through probe rpoB3 |ΔTm|. Ser450Leu was distinguished from Ser450Trp by probe rpoB4A |ΔTm|. The indeterminate result associated with His445Arg may be caused by its |ΔTm|=1.8°C compared with |ΔTm| typically exceeding 2°C for other mutations. Our recent experience with Ultra on diagnostic sputum samples only pertained to the Ser450Leu and His445Asp mutations, for which the ΔTm corresponded exactly with the ΔTm we observed on bacterial thermolysates. This should be validated more extensively, which is beyond the scope of our present study.

Conclusions

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

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disputed mutation. For such applications, it is key that ΔTm values are included in the exported results.

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78 References

79 1. Andre E, Isaacs C, Affolabi D, Alagna R, Brockmann D, de Jong BC, Cambau E,
80 Churchyard G, Cohen T, Delmee M, Delvenne JC, Farhat M, Habib A, Holme P,
81 Keshavjee S, Khan A, Lightfoot P, Moore D, Moreno Y, Mundade Y, Pai M, Patel
82 S, Nyaruhirira AU, Rocha LE, Takle J, Trebucq A, Creswell J, Boehme C. 2016.
83 Connectivity of diagnostic technologies: improving surveillance and accelerating
84 tuberculosis elimination. Int J Tuberc Lung Dis 20:999-1003.
85 2. Ng KC, Meehan CJ, Torrea G, Goeminne L, Diels M, Rigouts L, de Jong BC,
86 Andre E. 2018. Potential Application of Digitally Linked Tuberculosis Diagnostics
87 for Real-Time Surveillance of Drug-Resistant Tuberculosis Transmission:
88 Validation and Analysis of Test Results. JMIR Med Inform 6:e12.
89 3. Chakravorty S, Simmons AM, Rowneki M, Parmar H, Cao Y, Ryan J, Banada PP,
90 Deshpande S, Shenai S, Gall A, Glass J, Krieswirth B, Schumacher SG, Nabeta
91 P, Tukvadze N, Rodrigues C, Skrahina A, Tagliani E, Cirillo DM, Davidow A,
92 Denkinger CM, Persing D, Kwiatkowski R, Jones M, Alland D. 2017. The New
93 Xpert MTB/RIF Ultra: Improving Detection of Mycobacterium tuberculosis and
94 Resistance to Rifampin in an Assay Suitable for Point-of-Care Testing. MBio 8.
95 4. Van Deun A, Aung KJ, Bola V, Lebeke R, Hossain MA, de Rijk WB, Rigouts L,
96 Gumusboga A, Torrea G, de Jong BC. 2013. Rifampin drug resistance tests for
97 tuberculosis: challenging the gold standard. J Clin Microbiol 51:2633-40.
98 5. Rosser A, Marx FM, Pareek M. 2018. Recurrent tuberculosis in the pre-
99 elimination era. Int J Tuberc Lung Dis 22:139-150.

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Figure 1. Overview of Xpert Ultra test results. The observed probe reactions for each RRDR mutation were overlaid on claimed probe coverage (light gray). Shown in black are probe reactions concordant with manufacturer claims; in blue are probe reactions missed by one probe but captured by another probe; and in red is a probe reaction representing “RIF Resistance INDETERMINATE” result from 3 out of 4 strains tested. Results in striped pattern were superimposed for greater visibility.

Figure 2. Melting temperature shift (ΔTm) observed upon detection of a rifampicin resistance (RR)-conferring *rpoB* mutation in the rifampicin resistance determining region (RRDR) by Xpert Ultra. The y-axis reflects the melting temperature difference between mutant and wildtype probe-amplicon hybrids (ΔTm), while the x-axis shows the mutations we tested. The data points on the graph are ΔTm values grouped by associated Ultra probe (differentiated by color) corresponding with a specific *rpoB* 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 (ΔTm, 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 ΔTm 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	|ΔTm 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
*Met434Ile	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
*Ser441Gln	1	tcg > CAg	rpoB2; rpoB3	72.8-73.2; 73; 75.5-76.0; 75.75	68.3; 73.5	4.7; 2.3
*Ser441Leu	1	tcg > tTg	rpoB2; rpoB3		70.0; 73.5	3; 2.3

Thr444Thr	1	acc > acG	ND	ND	ND	ND
*His445Gln	1	cac > caG	rpoB3	75.5-76.0 | 75.75	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		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 | 75.75; 67.0-67.6 | 67.3	72.9-73.3; 73.3-73.8	2.5-2.9; 3.6-6.5
*Ser450Trp	3	tcg > tGg	rpoB3; rpoB4A		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
Ile491Phe	10	atc > Ttc	ND	ND	ND	ND
Ile491Val	1	atc > Gtc	ND	ND	ND	ND

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