Xpert MTB/XDR: a 10-Color Reflex Assay Suitable for Point-of-Care Settings To Detect Isoniazid, Fluoroquinolone, and Second-Line-Injectable-Drug Resistance Directly from *Mycobacterium tuberculosis*-Positive Sputum

Heta Parmar, [a] Yuan Cao, [a] Rajiv L. Gaur, [b] Deanna Lieu, [b] Shobana Raghunath, [b] Nova Via, [b] Simone Battaglia, [c] Daniela M. Cirillo, [c] Claudia Denkinger, [d*] Sophia Georghiou, [d] Robert Kwiatkowski, [b] David Persing, [b] David Alland, [a] Soumitesh Chakravorty[a],[b]

a New Jersey Medical School, Rutgers University, Newark, New Jersey, USA b Cepheid Inc., Sunnyvale, California, USA c San Raffaele Hospital, Milan, Italy d Foundation for Innovative New Diagnostics, Geneva, Switzerland

BACKGROUND

Drug-resistant *Mycobacterium tuberculosis* (MTB) remains a significant threat to global tuberculosis (TB) care and public health. With estimated 4.1% of new cases and 19% of previously treated cases had rifampicin-resistant TB (RR-TB) or multidrug-resistant TB (MDR-TB), and as many as 6.2% of MDR cases had extensively drug-resistant TB (XDR-TB) by WHO in 2018. Phenotypic drug susceptibility testing (pDST), the current gold standard for identifying drug resistance in MTB, takes 6 to 8 weeks to provide definitive results. Thus, treatment is often empirically based on other factors such as past medical history or local prevalence of resistance. Delays in appropriate treatment can increase both mortality and transmission of drug-resistant strains.

ASSAY INFORMATION

The Xpert MTB/XDR is a 2-phase, 10-color point-of-care molecular diagnostic assay that utilizes sloppy molecular beacons probes that target 8 different MTB genes detecting resistance to Isoniazid, Ethionamide, Fluoroquinolones and second-line injectable drugs and has microfluidics very similar to Xpert MTB/RIF assay.

Drugs	Probe	Target gene	Target codons/bp
	BG	Assay control	
Isoniazid	inhA	*inhA promoter*	-8 to -15
	katG	*katG*	315
	FabG1	*FabG1*	203
	ahpC	*OxyR-ahpC*	-48 to -6
Ethionamide	inhA	*inhA promoter*	-8 to -15
Fluoroquinolones	gyrA1	*gyrA*	88-94
	gyrA2	*gyrA*	88-94
	gyrA3	*gyrA*	88-94
	gyrB2	*gyrB*	538-540
Amikacin	rrs	16S rRNA	1400-1401
	eis	*eis promoter*	-14
Kanamycin	rrs	16S rRNA	1400-1401
Low level Kanamycin	eis	*eis promoter*	-8 to -14, -37
Capreomycin	rrs	16S rRNA	1400-1401

RESULTS

3-Tm pattern to distinguish low-resistance *gyrA* mutations

define output below — type genotypes and corresponding Tms below

Shift gyrA 94A-2 Tm by	0
Shift gyrA2-LR Tm by	0
Shift gyrA-VG1 Tm by	0
gyrA 94A-2 window	
Mut A	77.6	80
WT	73.2	77.5
Mut B	70	73.1
Mut C	59	69.9
gyrA2-LR window	
Mut A	72.1	80
WT	68.7	72
Mut B	58	68.6
gyrA-VG1 window	
Mut A	78.5	80.5
Mut B	74	77.8
WT	68.9	73
Mut C	55	68.8
100 windows		

output	94A-2	2-LR	VG1
S	WT	WT	WT
R	WT	WT	Mut C
R	WT	WT	Mut B
R	WT	WT	Mut A
ID	WT	WT	ID
R	WT	Mut B	WT
R	WT	Mut B	Mut C
R	WT	Mut B	Mut B
R	WT	Mut B	Mut A
R	WT	Mut B	ID
R Low	WT	Mut A	WT
R	WT	Mut A	Mut C
R	WT	Mut A	Mut B
R	WT	Mut A	Mut A
R	WT	Mut A	ID
ID	WT	ID	WT
R	WT	ID	Mut C

Sample Information					XDR Results			
Genotype	gyrA 94A-2	gyrA2-LR	gyrA-VG1	Resistance	Resistance	gyrA 94A-2	gyrA2-LR	gyrA-VG1
WT	76.1	70	70.8	S	S	WT	WT	WT
G88C	72.7	65.2	66.4	R	R	Mut B	Mut B	Mut C
G88A	71.7	63.9	65.4	R	R	Mut B	Mut B	Mut C
A90V	72	75.6	76.2	R Low	R Low	Mut B	Mut A	Mut B
S91P	72.2	74.8	66.1	R Low	R Low	Mut B	Mut A	Mut C
D94A	78.8	73.4	71.4	R Low	R Low	Mut A	Mut A	WT
D94G	76	69.5	75.8	R	R	WT	WT	Mut B
D94N	72.9	66.1	68.9	R	R	Mut B	Mut B	WT
D94Y	72.5	65.1	68.6	R	R	Mut B	Mut B	Mut C
D94H	73.2	65.6	68.9	R	R	WT	Mut B	WT
A90V, S91P	67.5	79.3	71.7	R	R	Mut C	Mut A	WT
A90V, G88C	67.8	71.3	72.2	R	R	Mut C	WT	WT
A90V, G88A	66.9	70.2	71	R	R	Mut C	WT	WT
A90V, D94G	71.9	75.4	80.2	R	R	Mut B	Mut A	Mut A
A90V, D94N	68		74.3	R	R	Mut C	ID	Mut B
A90V, D94A	75	78.6	76.8	R	R	WT	Mut A	Mut B

Figure. 1: Xpert MTB/XDR assay can distinguish A90V, S91P and D94A mutations that confer low-level resistance to fluoroquinolones from others associated with higher-level of resistance within gyrA gene by the virtue of Tm signature

Up to 20% of resistant population can be detected in a mixture

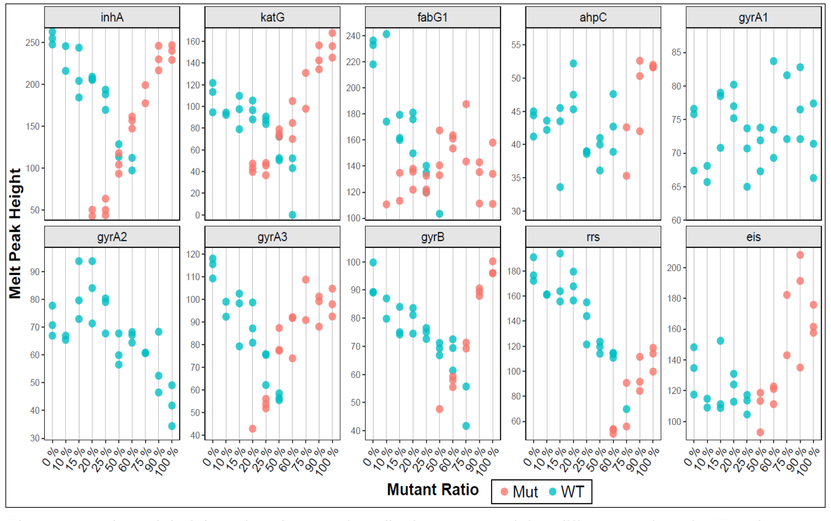
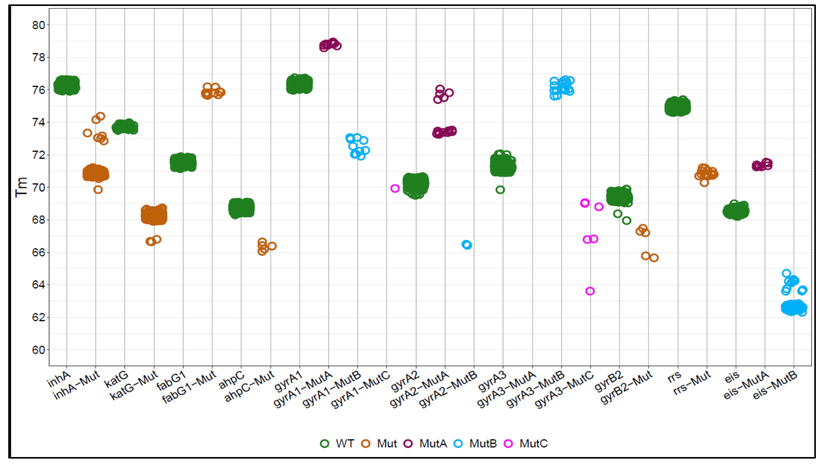


Figure. 2: Melt peak heights of each target in cell mixtures containing different ratios of WT and mutant plasmids respectively, where blue dots indicate a susceptible call and red dots indicate mutant calls based on their Tm and melt peak height. The melt peak height is determined by highest distance between the peak of the first derivative melt curve and baseline. The presence of blue and red dots for any concentration designates detection of both a WT and a mutant Tm. The QRDR mutation D94G generates a mutant Tm only with the gyrA3 probe.

High concordance with sequencing in a clinical trial

	pDST								
Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95%CI	Specificity (%)	95%CI
INH	309	284	5	19	1	98.3	95.8-99.3	95	73.1-99.7
FLQ	305	32	3	266	4	91.4	78.9-98.9	98.5	95.9-99.5
AMK	303	20	2	278	3	91	69.4-98.4	98.9	96.6-99.7
KAN	306	101	2	197	6	98.1	92.5-99.7	97.4	93.4-98.8
CAP	305	14	6	284	1	70	45.6-87.2	99.7	97.7-99.9
ETH	265	102	54	106	3	65.4	57.3-72.7	97.3	91.6-99.3
	Sequencing								
Drugs	N	TP	FN	TN	FP	Sensitivity (%)	95%CI	Specificity (%)	95%CI
INH	310	286	1	23	0	99.6	97.8-99.9	100	82.2-100
FLQ	309	39	1	269	0	97.5	85.3-99.8	100	98.2-100
AMK	306	24	0	282	0	100	82.8-100	100	98.3-100
KAN	308	109	4	195	0	96.4	90.6-98.9	100	97.6-100
CAP	307	16	1	290	0	94.1	69.2-99.6	100	98.4-100
ETH	310	108	14	183	5	88.5	81.2-93.4	97.3	93.6-99.0

Clustering of WT and mutant Tm from the clinical trials



SIGNIFICANCE

- Xpert MTB/XDR is designed as a reflex test for a specimen that is determined to be MTB positive, and optimize RR/MDR-TB treatment
- Rapid screening for resistance to multiple drugs simultaneously..
- Rapid fluoroquinolone resistance detection is critical, given its pivotal role in protecting bedaquiline against emergence of resistance in the new short-term treatment course.

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