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▶ To cite this version:

Bernice J. Klotoe, Natalia Kurepina, Elena Zholdibayeva, S. Panaiotov, Barry N. Kreiswirth, et al.. NTF-RINT, a new method for the epidemiological surveillance of MDR Mycobacterium tuberculosis L2/Beijing strains. Tuberculosis, 2020, 120, pp.101894. 10.1016/j.tube.2019.101894. hal-02502306

HAL Id: hal-02502306 https://hal.science/hal-02502306

Submitted on 21 Jul2022

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NTF-RINT, a new method for the epidemiological surveillance of MDR L2/Beijing strains Mycobacterium tuberculosis

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15 Keywords : atypical Beijing; typical Beijing; MDR-TB; molecular epidemiology

16

17 Abstract

The most widely discussed antibiotic-resistant tuberculosis strains ("W" and "B0/W148", "CAO") belong to L2/Beijing Lineage and are characterized by IS*6110* insertion sequences at the NTF locus. We present a high-throughput, microbead-based method, called NTF/RINT for detection of IS in <u>NTF</u> and <u>Rifampicin and Isoniazid Typing</u>. This method provides tuberculosis diagnostic confirmation, screens for the so-called modern L2/Beijing sublineage and detects mutations involved in resistance to Rifampicin (RIF) and Isoniazid (INH).

24 1. Introduction

Mycobacterium tuberculosis L2/Beijing is responsible of around one fifth of tuberculosis 25 cases around the world with the highest prevalence in Asia (50%) and Eastern Europe 26 (27.9%). In contrast, it is rare in South America and in Africa except for African coastal areas 27 and South Africa [1]. The L2/Beijing lineage harbors many IS6110 copies and most strains 28 have a specific spoligotyping profile: absence of spacers 1-34 and presence of most of 35-43 29 spacers in the CRISPR region previously referred to as DR region [2]. The L2/Beijing is 30 subdivided in two major groups based on the IS6110 copy numbers in a region usually 31 referred to as NTF region (between Rv3128c and Rv3129 genes, position 3494463-3493908 32 in H37Rv AL123456.3, GI:444893469) : "modern" (also referred to as "typical") and 33 "ancient" (also referred to as "atypical") Beijing strains [3]. 34

35 Some of the largest most successful tuberculosis clusters belong to the so-called modern 36 Beijing sublineage characterized by 1) at least one IS6110 insertion sequence in the 3' part of NTF region taken in its original orientation (Fig. 1), and 2) two mutations in 3R genes (mutT2 37 and ogt respectively at positions 58 and 12) [4]. The modern sublineage occurs worldwide 38 probably as a result of massive population migration, different immunogenic properties linked 39 to higher virulence [5, 6] and/or faster progression from latent infection to active tuberculosis 40 [7]. One of the reason for this increased virulence could be linked to PPE38 deletion, a 41 genotype preventing the secretion of several antigens that was found solely in modern Beijing 42 strains [8]. One of the first multi-drug resistant (MDR) epidemics reported was caused by a 43 modern Beijing strain termed "W", characterized by an IS6110 insertion in the 5' end of NTF 44 locus in addition to the one on the 3' end characteristic of modern sublineage [9]. More recent 45 L2/Beijing MDR outbreaks involve other modern isolates in Europe/Russia (B0/W148, 46 VNTR 100-32), and central Asia (CAO, VNTR 94-32) [10-12]. 47

In this study, we aimed at developing a new high throughput technique to 1) diagnose 48 49 tuberculosis detecting IS6110 insertion, 2) classify different Beijing genotype strains by typing IS6110 insertion in the NTF locus, and 3) identify drug resistance markers. We 50 previously developed the TB-SPRINT technique on Luminex technology that targets main 51 resistance mutations associated with Rifampicin (RIF) and Isoniazid (INH) resistance 52 53 simultaneously with spoligotyping (TB-SPRINT=TB-SPOL+TB-RINT, i.e. 43-Plex +16-Plex [13]. We propose here "NTF/RINT" a technique that combines RINT to the detection of 54 IS6110 in the NTF region. With this new technique, users will be able to classify strains in 55

different L2/Beijing clusters according to genotype and resistance profiles. This may
contribute to the improvement of the L2/Beijing epidemiological surveillance.

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59 2. Material and Methods

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2.1. Material origin and characterization

This study relies on two sets of strains. Forty-seven (n=47) clinical strains from New 61 York City (NYC), USA, were used to set up the method (sample set #1). Eighteen of them 62 belonged to the first large historical "W" MDR outbreak reported in NYC; double-checked 63 reference genotypes and partial phenotype characterizations were available for all these 64 strains. They were extracted by Cetyl-Trimethyl-Ammonium Bromide (CTAB) method as 65 described previously (van Soolingen, 1995). A second set (sample set #2) of 142 L2/Beijing 66 clinical strains from Kazakhstan was used as a validation set. Spoligotype pattern and full 67 genotype prediction as characterized by two methods (TB-SNPID, TB-SPRINT) were 68 69 available [14]. DNA was extracted by standard thermolysis on loops of solid cultures.

Genotype information concerning the IS*6110* insertions in the NTF locus for the set #1 was independently confirmed on agarose gel using slightly amended Plikaytis procedure: IS60 probe was changed to IS60B = 5' TGATGTGCTCCTTGAGTTCG 3' to amplify a 355 bp fragment [9]. For set #2, it was indirectly studied by specific mutation at amino acid 58 in *mutT2* included in TB-SNPID assay [15]. Genotype information concerning resistance loci was obtained by TB-SNPID and by TB-SPRINT [13]. BCG Paris strain and a sample from *Salmonella enterica* called "Sara8" were used as negative controls.

77 2.2. NTF/RINT principle, primers and, probes

For the IS6110 typing in NTF, regarding amplifications, we used previously published 78 79 primers for detecting IS6110 sequence [16] and designed new primers to amplify fragments overlapping NTF insertion sites: NTF5'_Fw: GGCTGCCTACTACGCTCAAC; NTF5'_Rv1: 80 TCGACAACCCACTTCCCATG; 81 NTF3'_Fw_new: TCACCACTGCTGCGTAATTCG; NTF3'_Rv_new: CCTGACATGACCCCATCCTTTC. For detection, we used a published 82 oligonucleotide sequence for IS6110 sequence [17] and designed sequences straddling NTF 83 IS6110 and sequences detect targeted insertions: NTF5'-probe 84 to the (GGCGGTTCAACATGGTGAC); NTF3'-probe (ATGAGGTCGTGAACCGCC). Probes 85 were coupled to microbeads as recommended by the manufacturer (www.luminex.corp). 86

The RINT part of NTF/RINT targets the same SNPs as those described by Gomgnimbou et al. (2013): 1) the *rpoB* mutations 435GTC, 445GAC, 445TAC, 450TTG, 450TGG; 2) the *katG* mutations 315ACC and 315AAC; 3) the inhA promoter mutations -15T and -8A. Please note that *rpoB* mutations are numbered according to their position in *M*. *tuberculosis* H37Rv strain annotation GenBank **AL123456.3**, GI:444893469, in contrast with previous studies referring to *E. coli* positions [13].

All primers and probes were obtained from integrated DNA Technologies (IDT,
Leuven, Belgium). The two spanning probes present in the TB-SPRINT assay development
were not included here.

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7 2.3. Amplification and detection on Luminex

The amplification reaction mixture contained 2μ L of DNA and 23 μ L of reaction mixture (0.2 mM dNTPs, 1 μ M mix of primers, 0.25U GoTaq Flexi, 1.5mM MgCl2, 1X GoTaq buffer) as recommended by the manufacturer (Promega Corp., Fitchburg, WI). Cycles consisted in 3 min at 96°C and 35 cycles of 30s at 96°C, 30s at 60°C, and 30s at 72°C. The rest of the procedure was performed as for TB-SPRINT [13] and hybridized microbeads samples were analyzed on a Luminex 200® device (Luminex, Austin, TX).

104 *2.4. Data interpretation*

105 Median Fluorescent Intensities (MFI) reported by the Luminex 200® device were analyzed as follows: for the presence/absence of IS6110 and its insertions in NTF region, the 106 107 standard cut-off for presence (MFI=150) was used and adjusted when necessary based on control samples values. For SNP genotyping in rpoB, katG genes and inhA promoter, the 108 109 genotypes were inferred as described in Klotoe et al (2018) using allele-call method and 110 maximal MFI [18] except when the DNA concentration was very low as evidenced by low signals for all markers, or rare erroneous inference in some of the controls due to high 111 background (inhA promoter -8A probe and rpoB 435GTC probe). In those cases, 112 interpretation was manual. 113

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115

116 **3. Results**

117 *3.1. NTF/RINT test design*

118 NTF/RINT test aims to identify likely epidemic MDR L2/Beijing strains through 119 targeting IS6110 sequence, its insertion at two specific locations in the NTF locus and the 120 mutations most frequently implicated in RIF and INH resistance (**Fig. 2**). Raw data are 121 Median Fluorescent Intensities (MFI) obtained through a Luminex platform that after 122 interpretation are converted to positive or negative values (black/white squares in **Fig. 2B**, 123 **Supplementary Material 1**).

124 *3.2.* Sensitivity and specificity on a reference laboratory sample

Applied on the reference sample set, NTF/RINT produced bimodal MFI values allowing 125 126 us to discriminate positive from negative signals. Genotypes were inferred accordingly. For the katG 315 position, no allele produced a positive signal for NYC outbreak strains in 127 128 accordance with them carrying a double mutation (AGC>ACA; Supplementary Material 1; Table 1). Three other genotypes were declared Not Determined (ND) although reference 129 130 method declared them "wt". A single allele of one strain was discordant with the reference method TB-RINT (locus rpoB 450 for strain 1595). Altogether, 347 over 376 loci were 131 132 assigned a genotype and 346 were concordant with the reference (99.7%). The method detected the 18 outbreak strains, 18 modern L2/Beijing and 11 strains belonging to ancient 133 Beijing clade or to other lineages, and a total of 24 MDR (Table 1). 134

135 *3.3. Evaluation on clinical strains from Kazakhstan*

136 A set of 142 Beijing clinical strains from Kazakhstan independently characterized with TB-

137 SPRINT and TB-SNPID methods were subjected to NTF/RINT. All but one (n=141) were

detected as modern Beijing by NTF/RINT as compared to 142 as detected by the reference

139 methods (*mutT2* mutation at position 58 detected by TB-SNPID). Sensitivity to detect modern

140 L2/Beijing was thus 99.6% while specificity could not be evaluated due to the composition of

141 the sample (absence of ancient L2/Beijing). No strains were detected that would carry solely

- 142 an insertion at the 5' end of the NTF locus.
- 143 Regarding the detection of resistance mutations, 130 strains (91.5%) and 980 loci out of 994
- 144 data points (98.6%) retrieved genotypes in full accordance with the reference
- 145 (Supplementary Material 1). A total of 101 isolates were correctly predicted as MDR-
- 146 modern Beijing, corresponding to a specificity of 93.5% (**Table 2**). Thirty out of a total of 34

strains predicted to be non-MDR were detected as such by NTF/RINT (sensitivity=88%).

148 MDR prediction could not rely on chance (Cohen's Kappa =0.81).

149

150 4. Discussion and conclusion

We set up NTF/RINT method to follow-up resistant Beijing strains by identifying most common RIF and INH resistance mutations and by identifying IS*6110* insertions in the NTF locus. Many IS*6110* insertions have been described in Beijing strains but only the one inserted on the 3' end of NTF has been linked to a group of clinical relevance, namely "modern Beijing" sublineage. The NTF-RINT assay is directly useful to tuberculosis researchers interested in bridging current standards to the more classical test set up by Plikaytis. The resulting assay is both sensitive and specific for all the mutations targeted.

Altogether in this study, NTF/RINT identified 125 modern L2/Beijing MDR strains, among which 18 belonged to the "W"-strain branch (from the NYC outbreak), and four MDR ancient Beijing. We identified a total of sixteen clusters according to genotypes (10 of which are represented in **Fig. 2**), and seven clusters regarding classification and phenotype: 1) MDR W-strain modern Beijing (n = 18), 2) MDR modern Beijing other than W (n = 108), 3) INH^R modern Beijing (n = 18), 4) susceptible modern Beijing (n = 29), 5) susceptible ancient Beijing (n = 7), 6) MDR ancient Beijing (n = 4), 7) INH^R ancient Beijing (n=1).

Regarding Beijing classification detection and sublineage prevalence, the dominance of 165 modern strains matches previous observations with a low prevalence of ancestral Beijing 166 except in Asia and South-Africa [10, 12, 19]. We detected no ambiguous cases where strains 167 would carry solely an insertion at the 5' end of the NTF locus. This was also true for the 168 L2/Beijing subsets from Peru, Mozambique, Bulgaria, Pakistan (data not shown). Merker et al 169 170 (2015) have reported L2/Beijing strains with this type of insertion but their analysis reported any type of insertion whereas NTF/RINT targets only sequences straddling over IS6110 at the 171 exact position described by Plikaytis et al (1994). The large study by Shitikov et al. also 172 observed no such case, and in contrast, identified a perfect link between the IS6110 insertion 173 at the 3' end of NTF and the affiliation to the modern subbranch of Beijing [4]. Concerning 174 "W"-strain subbranch, its typical signature was detected solely in samples from the USA. This 175 matches its historical localisation and indicates no spreading of the epidemics. 176

177 Regarding resistance levels, we detected a very high prevalence of resistance among
178 modern Beijing strains as described on the complete study on Kazakhstan [14]. These strains
179 actually belong to the already well-known 94-32 epidemic cluster.

Limitations of this test in detecting MDR result from the detection of only the most 180 181 common antibiotic resistance mutations. We screened RIF Resistance due to 445 and 450 rpoB mutations that have shown to represent 80-91% of all RIF Resistance and INH 182 resistance due to katG 315ACC and 315AAC mutations responsible for 70% of INH 183 resistance [13]. Noteworthy, mutations other than the targeted ones lead to low signals 184 185 causing either indeterminations or erroneous assignation. As an example, the single case of discordance in the set #1 which concerned rpoB 450 locus correlated with the 452CCG 186 187 mutation also conferring resistance. Altogether, NTF/RINT test may be useful for first-line screening for any first-line drugs resistance [15]. 188

As compared to other genotyping methods predicting resistance and/or classification, 189 NTF/RINT is relatively cost-effective (~4.5 euros of reagents for our laboratory in France in 190 2017-2019 including expenses for controls) due to the use of a restricted number of only 17 191 targets and the absence of need for the more costly xTAG® Luminex microbeads as used by 192 TB-SNPID [15]. Another characteristic of this technique is its high throughput since in one 193 working day, 90 samples can be processed. In contrast, testing smaller batches of samples 194 195 with this high-throughput method does not reduce turn-around time and increases the cost per sample as the same number of controls is needed irrespective of the number of samples. This 196 test thus rather suits batch testing for retrospective studies for exploration or quality-control. 197 198 This test is best suited to settings where the circulating MDR-Beijing belong to several of the subgroups detected by the test (W-strains, modern Beijing, ancient Beijing). This is the case 199 200 mainly in USA, South-Africa, Pacific region, former Soviet Union countries, and even more in China where ancient- Beijing varies between ~10% to more than 44% [18]. In that case, 201 202 our method reaches a high discriminatory power for a very high throughput and low cost. It 203 can also serve as a control for species identification and check for resistance mutation 204 detection. The robustness of the test in trained hands makes it a priori suitable to further uses of cartridges DNA remnants as can be retrieved from Genexpert's to confirm and/or refine 205 206 screening [20]. Studies measuring sensitivity and reproducibility in different settings to evaluate robustness of the whole protocol should be performed before large scale 207 implementation. In addition to providing the detection of IS insertions in NTF, this test serves 208 as a proof-of-concept for a new type of assay as compared to SNP or spacer detection 209

previously set-up on microbead-based technique, namely the detection of insertions or deletions. This concept may later serve detecting other types of insertions/deletions, such as deletion of PE-PPE genes involved in modern Beijing strains virulence [20]. The NTF-RINT test is also meant to be expanded to a 50-Plex IS*6110* and SNPs-based method dedicated to L2 genotyping. This second step remains pending.

215

216 Acknowledgements

François-Xavier Weill is acknowledged for giving Salmonella DNA sample Sara8 to serve as
negative control for IS*6110* detection. Sarah Sengstake is acknowledged for contribution to
the genotyping of set #2 by TB-SNPID method.

220

221 Conflict of interest

Bernice Klotoe was partly founded by Beamedex®, a society that sells microbead-based kits. The society had however no part in the decision of what new tests could be set-up and this was entirely decided by public researchers Christophe Sola, Guislaine Refrégier, with the consent of Natalia Kurepina and Barry N. Kreiswirth. Guislaine Refrégier acknowledges one travel grant by Luminex® on another project regarding SNP detection. Luminex® is a manufacturer of microbead-based instruments and microbeads.

228

229 Figure legends

230

Figure 1- NTF structure showing IS6110 positions and orientations compared to H37Rv
reference genome (AL123456.3, GI:444893469).

233

Figure 2 - Evolutionary scenario of IS6110 in NTF the locus of Beijing Strain (A) and corresponding identified clusters according to allele presence/absence (B).

236

237 Table legends

- Table 1- Concordance between NTF/RINT and controls on the reference samples set.
- Table 2- Prediction of modern MDR Beijing on a Kazakhstan sample by NTF/RINT versus
 TB-SPRINT/TB-SNPID

241



Figure 1 : NTF structure showing IS6110 positions and orientations compared to H37Rv reference genome.

A	156110		В	IS6110	IS6110:: 5'NTF	IS6110:: 3'NTF	rpoB_435_Wt	rpoB_435_mut_GTC	rpoB_445_Wt	rpoB_445_mut_GAC	rpoB_445_mut_TAC	rpoB_450_Wt	rpoB_450_mut_TTG	rpoB_450_mut_TGG	katG_315_Wt	katG_315_mut_ACC	katG_315_mut_AAC	inhA15_wt	inhA 15_mut_T	inhA_8_mut_A
		W strain Modern /typical L2/Beijing	W-strain																۵	
3' NTF insertion	NTF region		Most frequent MDR modern Beijing strain Other MDR modern Beijing strain	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•	•
IS6110_			Susceptible modern Beijing strain	•		•	•	٥	•	۵	۵	•	٥		•	۵		•	۵	
insertions	NTF region	Ancient /atypical L2/Beijing	Most frequent ancient Beijing (susceptible) strain Rare MDR ancient Beijing strain	•	0	•	•	•	•	•	•	•		•	•			•	0	•
	NTF region 556 bp	Other TB lineages	Other lineage MDR strain Other lineage suseptible strain	•	•	•	•	•		•	0		•	•	•	•	•	•	•	•
\		Non-TB bacteria	non-MTC strain	۵	٥		•	٥	•	٥	٥	•	٥	٥	•	٥	۵	۵	۵	•

Figure 2 : Evolutionary scenario of IS6110 in NTF the locus of Beijing Strain (A) and corresponding identified clusters according to allele presence/absence (B).

N°	#	NY NTF/RINT profiles Control profiles								
Out	b.	IS 611	0 profile	R. loc. profile	GDST	IS6110 on gel	TB_RINT or seq	PhDST		
1	2015	YES	2 IS <i>6110</i> ::NTF	435?/445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
2	2706	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
3	3452	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
4	3267	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
5	6138	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
6	8121	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
7	16852	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
8	1582	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
9	2470	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
10	15183	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
11	3542	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
12	4953	YES	2 IS <i>6110</i> ::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
13	19556	YES	2 IS6110::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
14	13223	YES	2 IS6110::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
15	19996	YES	2 IS6110::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
16	1858	YES	2 IS6110::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	MDR		
17	19962	YES	2 IS6110::NTF	445TAC/315?	MDR	2 IS <i>6110</i> ::NTF	445TAC/315C	ND		
18	13250	YES	2 IS6110::NTF	445TAC/315?	R ^R	2 IS <i>6110</i> ::NTF	445TAC/315C	ND		
19	11455	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	SUSC		
20	14031	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	SUSC		
21	14907	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	SUSC		
22	15803	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	SUSC		
23	16377	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	SUSC		
24	7716	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
25	7718	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
26	20935	NO	1 IS <i>6110</i> ::NTF	445?/WT	ND	1 IS <i>6110</i> ::NTF	WT/WT	ND		
27	12783	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
28	6595	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
29	20251	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
30	20217	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	ND		
31	17316	NO	1 IS <i>6110</i> ::NTF	WT/WT	SUSC	1 IS <i>6110</i> ::NTF	WT/WT	S ^R		
32	15550	NO	1 IS <i>6110</i> ::NTF	450TTG/-15T	MDR	1 IS <i>6110</i> ::NTF	450TTG/-15T	ND		
33	16870	NO	1 IS <i>6110</i> ::NTF	450TTG/315C	MDR	1 IS <i>6110</i> ::NTF	450TTG/315C	ND		
34	3219	NO	1 IS <i>6110</i> ::NTF	WT/315C	^R	1 IS <i>6110</i> ::NTF	WT/315C	SI ^R		
35	10598	NO	1 IS <i>6110</i> ::NTF	450TTG/315C	MDR	1 IS <i>6110</i> ::NTF	450TTG/315C	SIE ^R		
36	11877	NO	1 IS <i>6110</i> ::NTF	435?/315C	ND/ I ^R	1 IS <i>6110</i> ::NTF	WT/315C	ND		
37	12766	NO	IS6110	WT/WT	SUSC	IS6110	WT/WT	SUSC		
38	12360	NO	IS6110	WT/WT	SUSC	IS6110	WT/WT	SUSC		
39	20082	NO	IS6110	WT/WT	SUSC	IS6110	WT/WT	ND		
40	22661	NO	IS6110	WT/WT	SUSC	IS6110	WT/WT	ND		
41	33452	NO	IS6110	WT/WT	SUSC	IS6110	WT/WT	ND		
42	18842	NO	IS6110	435?/WT	ND/WT	IS6110	WT/WT	ND		
43	12551	NÔ	IS6110	WT/WT	SUSC	IS6110	WT/WT	ND		
44	10545	NÔ	IS6110	450TTG/315C	MDR	IS6110	450TTG/315C	MDR		
45	11265	NO	IS6110	450TTG/315C	MDR	IS6110	450TTG/315C	MDR		
46	1595	NO	IS6110	450TGG/315C	MDR	IS6110	WT/315C	ND		
47	4948	NO	IS6110	WT/315C	IR I	IS6110	WT/315C	ND		
<u></u>					· •					

Table 1- Concordance between NTF/RINT and controls on the reference samples set.

Outb=outbreak; GDST=Genotypic Drug Susceptibility Testing (DST); PhDST=Phenotypic DST; ND=Not Determined. I=Isoniazid; R=Rifampicin; S=Streptomycin; E=Ethionamide; SUSC=Susceptible; ^R/R.=Resistance; loc=locus. Seq=sequencing. The single true discordance is underlined.

Table 2- Prediction of modern MDR Beijing on a Kazakhstan sample by NTF/RINT versus TB-SPRINT/TB-SNPID

	Reference (TB-SNPID, TB-SPRINT, Plikaytis)							
	MDR-modern Beijing non-MDR modern Beijing Total							
NTF/RINT								
MDR-anc. Beijing	1	0	1					
MDR-modern Beijing	101	4	105					
non-MDR modern Beijing	6	30	36					
Total	108	34	142					

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