National Public Health Laboratory Drug Susceptibility Testing Reference Center for Mycobacterium tuberculosis Molecular Assay Performance Characteristics

California Department of Public Health
Microbial Diseases Laboratory (MDL) Branch

Genes and loci evaluated by MDL assays:

Gene name	Associated with resistance to	WGS Start nt position	WGS Stop nt position	tNGS Start nt position	tNGS Stop nt position
*rpoB-FL**	rifampicin	759607	763525	n/a	n/a
*rpoB-1**	rifampicin	n/a	n/a	760280	760812
*rpoB-2**	rifampicin	n/a	n/a	760957	761355
pncA	pyrazinamide	2288481	2290323	2288672	2289301
embA	ethambutol	4243004	4246717	*not tested*	*not tested*
embB	ethambutol	4246314	4250010	4247376	4248065
katG	isoniazid	2153689	2156570	2153404	2156137
fabG1	isoniazid, ethionamide	1673148	1674383	1673321	1673755
inhA	isoniazid, ethionamide	1673848	1675211	1674287	1674880
ethA	ethionamide	4325804	4330174	4325951	4327510
gyrB	moxifloxacin, levofloxacin	5040	7467	7377	7754
gyrA	moxifloxacin, levofloxacin	7102	10018	6298	6943
rrs	kanamycin, capreomycin, amikacin	1471646	1473582	1471850	1473945
eis	kanamycin, amikacin	2713924	2715586	2715171	2715528
tlyA	capreomycin	1917740	1918946	1917811	1918750
Rv0678	clofazimine, bedaquiline	778790	779687	778990	779487
mmpL5	clofazimine, bedaquiline	775386	778680	*not tested*	*not tested*
mmpS5	clofazimine, bedaquiline	778277	779105	*not tested*	*not tested*
pepQ	clofazimine, bedaquiline	2859100	2860618	*not tested*	*not tested*
atpE	bedaquiline	1460845	1461490	*not tested*	*not tested*
rplC	linezolid	800609	801662	801108	801483
rrl	linezolid	1473458	1476995	1475923	1476625

Footnotes: Position coordinates listed in relation to the M. tuberculosis H37Rv NC_000962.3
* WGS-DST assay sequences the full-length gene and promoter region of rpoB, while tNGS-DST assay covers two fragments within rpoB (including RIF-resistance determining region or RRDR)

Overall MTBC WGS-DST and tNGS-DST assays performance:

Performance Characteristic	Evaluated aspect of the assay	WGS	tNGS
Accuracy	**Predicted S/R profile**	98.16%	98.68%
	Detection of genomic variations in targeted loci	99.73%	99.70%
	MTBC ID	100%	96.88%
Repeatability (Qualitative)	**Predicted S/R profile**	100%	100%
	Detection of genomic variations in targeted loci	100%	100%
	MTBC ID	100%	100%
Reproducibility (Qualitative)	**Predicted S/R profile**	100%	100%
	Detection of genomic variations in targeted loci	99.80%	99.50%
	MTBC ID	100%	100%
Diagnostic Sensitivity	**Predicted S/R profile**	91.25%	93.44%
	Detection of genomic variations in targeted loci	99.00%	98.90%
	MTBC ID	100%	96.36%
Diagnostic Specificity	**Predicted S/R profile**	99.40%	100%
	Detection of genomic variations in targeted loci	99.89%	99.80%
	MTBC ID	100%	100%

Please Note: The validation datasets differed; a direct performance comparison between the two methods based on the values below is not possible.

Definitions:

- Predicted S/R profile- predicted susceptibility(S)/resistance(R) to drugs in MTBC organisms based on WGS-DST or tNGS-DST.
- Detection of genomic variations in targeted loci- detection of genomic variations (single nucleotide polymorphisms [SNPs], multi nucleotide polymorphisms [MNPs], and indels) throughout the established reporting range in the genetic targets included in this assay that are known or suspected of being responsible for drug resistance in MTBC.
- MTBC ID- For WGS: identification of MTBC (based on lineage-specific SNPs) and differentiation of *M. tuberculosis* from *M. bovis*, and further delineation of *M. bovis* BCG strain. For tNGS: ability to detect MTBC DNA from clinical specimen and differentiation of *M. bovis* vs. MTBC not-*M. bovis.*

WGS-DST assay performance by drug:

Drug	TP	TN	FN	FP	Accuracy	Dx Sensitivity	Dx Specificity	PPV	NPV
INH	85	86	2	0*	**98.84%**	**97.70%**	**100%**	100%	97.73%
ETA	44	114	0	3*	**98.14%**	**100%**	**97.44%**	93.62%	100%
RIF	18	171	0	2	**98.95%**	**100%**	**98.84%**	90.00%	100%
PZA	26	147	16	0	**91.53%**	**61.90%**	**100%**	100%	90.18%
EMB	11	170	0	3*	**98.37%**	**100%**	**98.27%**	78.57%	100%
AMK	5	173	0	0	**100%**	**100%**	**100%**	100%	100%
KAN	4	126	0	0	**100%**	**100%**	**100%**	100%	100%
CAP	6	183	1	0	**99.47%**	**85.71%**	**100%**	100%	99.46%
MFX	17	129	2	0	**98.65%**	**89.47%**	**100%**	100%	98.47%
LFX	1	14	0	0	**100%**	**100.00%**	**100%**	100%	100%
BDQ	0	5	0	0	**100%**	**N/A**	**100%**	N/A	100%
CFZ	0	5	0	0	**100%**	**N/A**	**100%**	N/A	100%
LZD	2	2	0	0	**100%**	**100%**	**100%**	100%	100%
Overall	219	1325	21	8	**98.16%**	**91.25%**	**99.40%**	96.48%	98.44%

Footnotes: *Numbers after discrepancies were resolved with gold standard method.

tNGS-DST assay performance by drug:

Drug	TP	TN	FN	FP	Accuracy	Dx Sensitivity	Dx Specificity	PPV	NPV
INH	13	16	1	0	**96.67%**	**92.86%**	**100%**	100%	94.12%
ETA	7	24	1	0	**96.88%**	**87.50%**	**100%**	100%	96%
RIF	10	27	0	0	**100%**	**100%**	**100%**	100%	100%
PZA	7	23	2	0	**93.75%**	**77.78%**	**100%**	100%	92.00%
EMB	8	28	0	0	**100%**	**100%**	**100%**	100%	100%
AMK	3	31	0	0	**100%**	**100%**	**100%**	100%	100%
KAN	2	19	0	0	**100%**	**100%**	**100%**	100%	100%
CAP	3	32	0	0	**100%**	**100%**	**100%**	100%	100%
MFX	4	26	0	0	**100%**	**100%**	**100%**	100%	100%
LFX	0	2	0	0	**100%**	**N/A**	**100%**	N/A	100%
BDQ	0	5	0	0	**100%**	**N/A**	**100%**	N/A	100%
CFZ	0	5	0	0	**100%**	**N/A**	**100%**	N/A	100%
LZD	0	4	0	0	**100%**	**N/A**	**100%**	N/A	100%
Overall	57	242	4	0	**98.68%**	**93.44%**	**100.00%**	100.00%	98.37%

Please Note: Sensitivity for PZA for both the tNGS and WGS assays is likely underestimated as the reference method (i.e. phenotypic DST by MGIT) is known to have a high rate of false resistance due to technical variation that may affect results. The sensitivity for fluoroquinolones in our study was likely impacted by the presence of heteroresistance.

WGS-DST assay performance by gene target:

Gene	TP	TN	FN	FP	Accuracy	Dx Sensitivity	Dx Specificity	PPV	NPV
pncA	27	157	0	0	100%	100%	100%	100%	100%
katG	48	123	0	0	100%	100%	100%	100%	100%
fabG1/ inhA	43	129	0	0	100%	100%	100%	100%	100%
rpoB	30	143	0	0	100%	100%	100%	100%	100%
gyrA	21	98	2	0	98.35%	91.30%	100%	100%	98.00%
gyrB	0	27	0	0	100%	N/A	100%	N/A	100%
rrs	5	114	0	0	100%	100%	100%	100%	100%
embB	5	23	0	0	100%	100%	100%	100%	100%
rv0678	12	5	0	0	100%	100%	100%	100%	100%
atpE	3	15	0	0	100%	100%	100%	100%	100%
pepQ	1	16	0	1	94.44%	100%	94.12%	50%	100%
mmpL5	1	15	0	0	100%	100%	100%	100%	100%
mmpS5	1	15	0	0	100%	100%	100%	100%	100%
rplC	2	16	0	0	100%	100%	100%	100%	100%
rrl	0	12	0	0	100%	N/A	100%	N/A	100%
Total	**199**	**908**	**2**	**1**	**99.73%**	**99.00%**	**99.89%**	**99.50%**	**99.78%**

Footnote: Performance for several WGS gene targets could not be calculated directly due to lack of reference molecular data on mutations in those loci.

tNGS-DST assay performance by gene target:

Gene	TP	TN	FN	FP	Accuracy	Dx Sensitivity	Dx Specificity	PPV	NPV
pncA	12	25	0	0	100%	100%	100%	100%	100%
katG	18	31	0	0	100%	100%	100%	100%	100%
inhA	2	59	0	0	100%	100%	100%	100%	100%
fabG1	7	45	0	0	100%	100%	100%	100%	100%
rpoB	15	39	0	0	100%	100%	100%	100%	100%
gyrA	6	36	0	0	100%	100%	100%	100%	100%
gyrB	5	37	0	0	100%	100%	100%	100%	100%
rrs	4	34	0	1	97.40%	100%	97.10%	80.00%	100%
eis	1	37	0	0	100%	100%	100%	100%	100%
tlyA	1	36	0	0	100%	100%	100%	100%	100%
embB	10	28	0	0	100%	100%	100%	100%	100%
rv0678	4	34	0	0	100%	100%	100%	100%	100%
rplC	1	36	0	0	100%	100%	100%	100%	100%
rrl	0	36	0	0	100%	N/A	100%	N/A	100%
ethA	7	30	1	0	97.40%	87.50%	100%	100%	96.80%
Total	**93**	**543**	**1**	**1**	**99.70%**	**98.90%**	**99.80%**	**98.90%**	**99.80%**

Assay Interferences & Limitations:

- Phenotypic DST:
    - Results may be delayed or unattainable if a mixed culture is submitted
- WGS:
    - Submission of culture on media types other than LJ or MGIT broth, or submission of cultures with scant growth, will delay testing and availability of results.
    - A negative result (i.e. no mutations detected) does not rule out the presence of resistance-conferring mutations elsewhere in the genome. Heteroresistance below the level of detection of this assay may also contribute to false-negative results.
- tNGS:
    - Inhibitory substances in the specimen may prevent successful DNA amplification and sequencing. MTBC tNGS-DST assay is not a presence/absence test. Inability to generate successful sequences in targeted drug-resistance associated loci or negative MTBC ID result do not preclude the presence of MTBC.
    - Absence of reportable mutations does not rule out the presence of resistance-conferring mutations elsewhere in the genome. Heteroresistance below the level of detection of this assay may contribute to predicted false-susceptible results.
- Sequencing-based DST test results should not be used as the sole criterion for the diagnosis of drug susceptible or resistant tuberculosis but can be used in conjunction with other clinical data.