



NATIONAL HEALTH
LABORATORY SERVICE

Validating Roche cobas 6800 MTB and MTB-RIF/INH assays for mycobacterial cultured isolates

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Background

- Roche cobas 6800 MTB and MTB RIF/INH added as NHLS screening platform for tuberculosis (TB) in 2024
 - 'Two step' testing : Roche cobas MTB for TB detection followed by MTB-RIF/INH for susceptibility if positive
 - Validated for raw sputum and processed sediments
- Only one platform currently within NHLS to test culture isolates:
GeneXpert MTB/RIF Ultra

Objectives

- Validate performance of Roche cobas MTB and MTB-RIF/INH assays to
 - identify *Mycobacterium tuberculosis* complex (MTBC) in cultured isolates
 - determine drug susceptibility to rifampicin (RIF) and isoniazid (INH)
- [characterised by whole genome sequencing (WGS)]

Methods (1)

- Stored isolates from NICD National TB Reference Laboratory previously characterised by WGS
- RIF *rpoB* mutations
- INH *katG* and *inhA* mutations } (n=30)
- Wilde-type isolates (n=30)

As negative samples/controls:

- Cultures with non-tuberculous mycobacteria (NTM) as determined by Hain GenoType Mycobacterium CM (n=30)
- GeneXpert MTB/RIF Ultra for discordant results between stored sample results and Roche (MTB detection and rifampicin susceptibility)

Methods (2)

- Testing on Roche instruments at Braamfontein NHLS Mycobacteriology Referral Laboratory, Johannesburg (as per manufacturer instructions) in Aug/Sep 2025
- 'Sediment' protocol
- Acceptance criteria for performance: 80% concordance

Results (1)

- Total samples processed n=96
 - MTBC positive n=64
 - Wilde type n=26
 - Rifampicin resistance mutations n=37
 - Isoniazid resistance mutations n=26
 - NTM n=32

Results (2)

Performance of Roche cobas MTB and MTB-RIF/INH assays for detection of MTB and susceptibility testing (rifampicin, isoniazid) in cultured isolates vs WGS/PCRCM

	n	TP	FP	TN	FN	Sensitivity	Specificity
MTB Detection	96	64	8	24	0	100% ✓	75% ✗
RIF-R Detection	63	32	0	26	5	86.5% ✓	100% ✓
INH-R Detection	64	23	1	37	3	88.5% ✓	97.4% ✓

Discordant results

- **MTB detection:**

NTMs identified as MTB on Roche cobas (n=8 FN):

- x5 *M. kansasii*
- *M. goodnae*, *M. scrofulaceum*, *M. szulgai*

[Xpert Ultra: x6 MTB not detected]

- **Susceptibility:**

- x5 rifampicin resistant isolates reported as susceptible (majority not covered by assay; x2 present at low frequency)
- x3 isoniazid resistant isolates reported as susceptible (as above)
- x1 isoniazid susceptible isolate reported as resistant

Conclusion

- Sub-optimal accuracy for MTB detection; however, lab flow of specimens unlikely to result in testing of NTM positive cultures on Roche (ie MPT64 antigen negative)
 - Roche cobas MTB detects *esx-1* gene present in both MTB and *M kansasii* genomes
 - Roche susceptibility testing for rifampicin and isoniazid acceptable (within 80% cut-off)
- Suggest national validation; further exploration of misidentified NTM spp