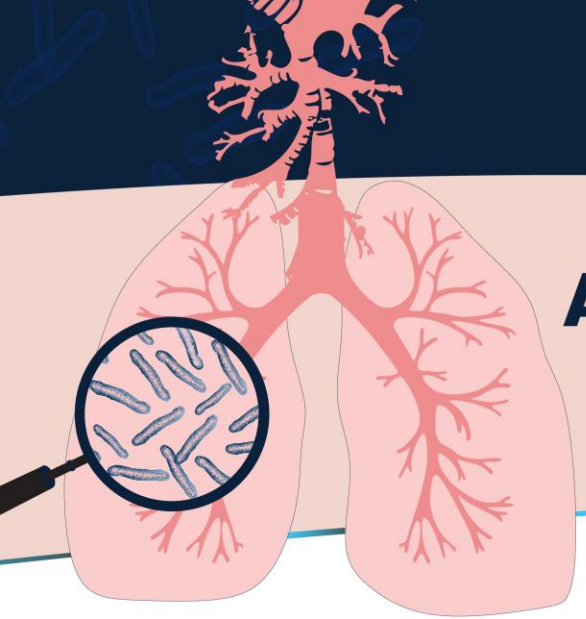

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Stability of *Mycobacterium Tuberculosis* in Xpert Sample Reagent for extended incubation and potential value for delayed follow-on testing on the Xpert MTB/XDR test in high throughput laboratories

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BACKGROUND

- The Cepheid Xpert MTB/RIF assays have been used programmatically in South Africa for the primary diagnosis of TB and rifampicin resistance
- As a follow-on test Cepheid developed the Xpert MTB/XDR (XDR) for additional drug-resistant detection to satisfy the WHO definitions criteria of MDR and XDR at the time to guide patient management.
- The World Health Organization recommended Xpert XDR assay as a low complexity rapid diagnostic in 2020.
- The National TB Programme in consultation with the National Health Laboratory Service made the decision to introduce this assay, thereby, replacing the line-probes assays used at the time.
- To improve turnaround times due to modular nature of the assay and reduced labour hands on time
- Cost saving potential

Drug	Gene Target
Isoniazid	*inhA promoter* *katG* *fabG1* *oxyR-ahpC intergenic region*
Ethionamide	*inhA promoter*
Fluoroquinolones	*gyrA* *gyrB*
Amikacin, Kanamycin, Capreomycin **Kanamycin ONLY**	*rrs* *eis promoter*

BACKGROUND

- Studies investigating the impact of prolonged incubation periods in SR by Helb *et al*., (2010) reported that the sample remained stable in SR buffer up to 5 days and the PCR cycle threshold (Ct) remained unaffected (without causing a significant delay) for at least 24 hours for the detection of MTB.

- Furthermore, Bananda *et al* (2021). study showed that prolonged incubation had no impact on the Xpert test sensitivity for at least 3 days.

- However, paucibacillary samples showed false resistance to RIF when incubated for longer than 24 hours. The mutations found were different from the ones usually conferred by clinically resistant samples suggesting that they were potentially induced by DNA deamination because of sodium hydroxide (Banada *et al*., 2010).

PROBLEM

- The sample inactivation procedure used in the assay contains sodium hydroxide and isopropanol to inactivate the bacteria and liquefy the patients' sputum.
- One of the limitations of the assay, in our setting, is that once the patient sample is combined with the pre-step sample reagent (SR) the manufacturer recommends that testing should occur with in 4 hours.
- It is hypothesized that keeping the sample in SR for longer may decrease sample stability and produce inaccurate results based on previous studies .

STUDY RATIONAILE AND AIM

AIM

- To evaluate the impact on stability with extended sample incubation beyond 4 hours in SR at selected time points and temperature conditions.

RATIONAILE

- Any improvement on the time to testing of the sample in SR, will give operators sufficient time to complete both tests form a single SR treated specimen. This will further reduce lab error and improve TAT providing clinicians with sufficient information to adequately manage patients' early on.

OBJECTIVES

1. To assess the stability of the sample in SR at different time intervals beyond the recommended 4-hour period.
2. To assess the impact of extended incubation on cycle-threshold and melt-temperature instrument read-outs.
3. To assess the stability of the sample in SR buffer at different temperature conditions, which include ambient laboratory temperature, refrigerated (4°C – 8°C) and frozen (-20°C) beyond the recommended 4-hour period

METHODS

- Phenotypically (DST) and genotypically (WGS) pre-characterized isolate panel (n=5) with known wild type and common mutations in the genes targeted by the Xpert MTB/XDR assay

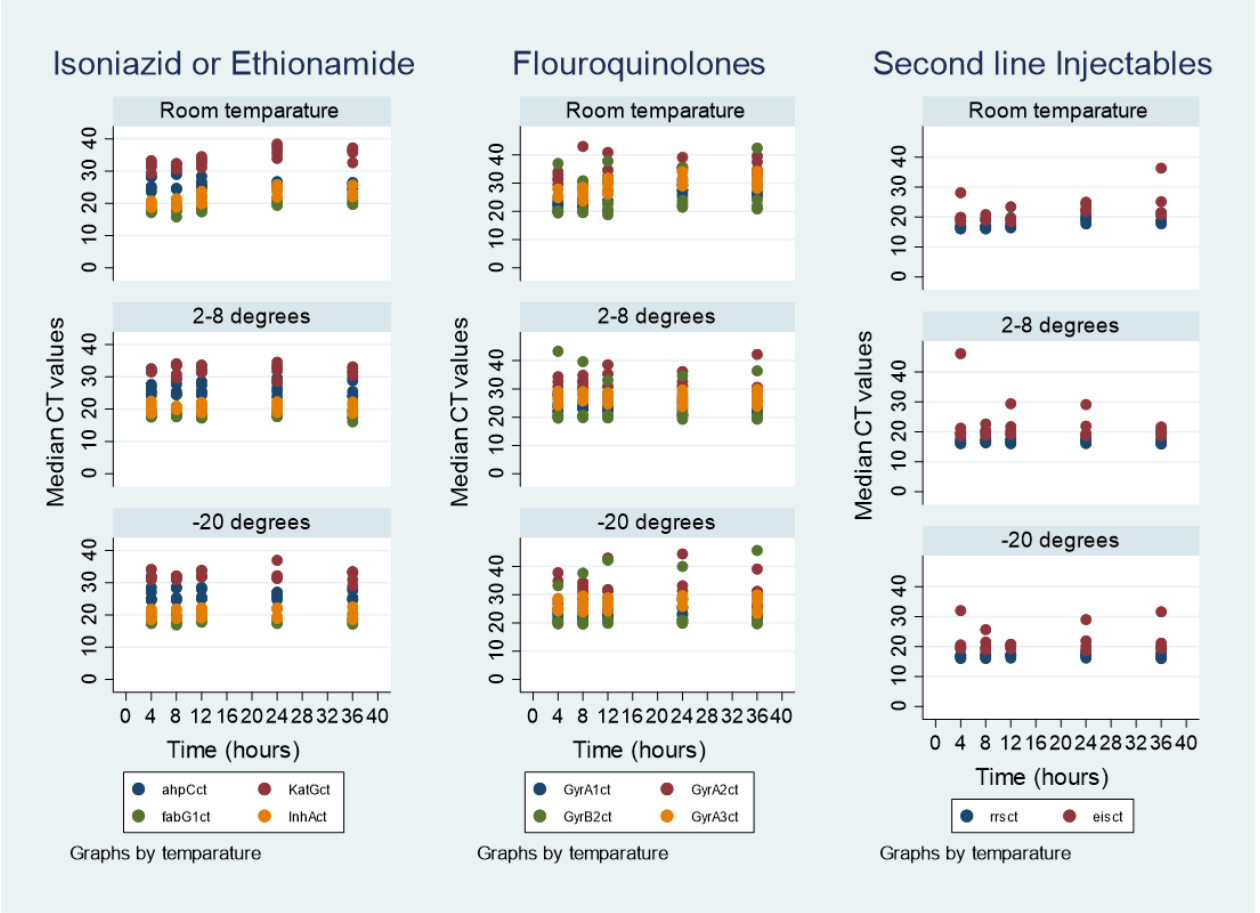
- To assess the impact of extended incubation on cycle-threshold and melt-temperature instrument read-outs.

- To assess the stability of the sample in SR buffer at different temperature conditions, which include ambient laboratory temperature, refrigerated (4°C – 8°C) and frozen (-20°C) beyond the recommended 4-hour period

#	inhA promoter	katG	gyrA	gyrB	rrs	eis promoter
1		SER315THR		GLU501ASP		
2		SER315THR	ASP94GLY	GLU501ASP		C-10T
3	G-17T	SER315THR	ASP94HIS	ALA504VAL	A1401G	
4	C-15T		ASP94GLY			
H37Rv						

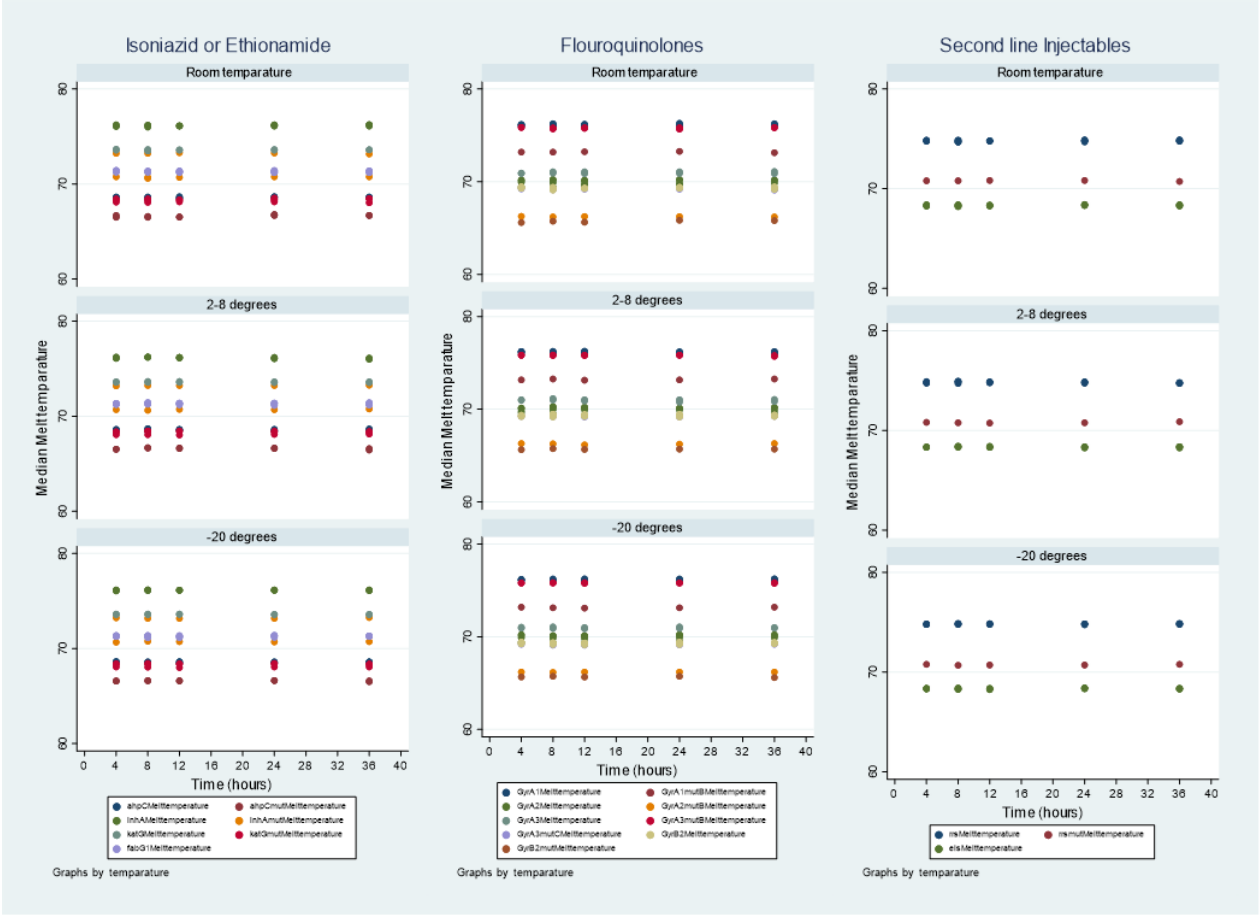
RESULTS – Cycle Threshold

- There was no significant difference in Cycle threshold (Ct) of analytes associated with isoniazid (INH), Ethionamide (ETH), Fluoroquinolones (FQ) and Second Line Injectables between the incubation periods 4, 8-, 12-, 24- and 36- hours at different temperatures RT, 4°C and -20°C was observed, p-value >0.05.



RESULTS – Melt Temperature

- There was no significant difference in melt temperature (Tm) of analytes associated with isoniazid (INH), Ethionamide (ETH), Fluoroquinolones (FQ) and Second Line Injectables between the incubation periods 4, 8-, 12-, 24- and 36- hours at different temperatures RT, 4°C and -20°C was observed, p-value >0.05.



CONCLUSIONS

The extended incubation of samples in SR was found to have notable deterioration on the test performance at all time points & temperatures ranges.

Susceptibility patterns for genes associated with drug resistance detection remained consistent across all conditions.

These finding suggests a potential solution to optimize drug resistance testing by reducing turnaround time equipping health care workers with the necessary information for adequate patient management.