NATIONAL INSTITUTE FOR COMMUNICABLE DISEASES
Division of the National Health Laboratory Service

Evaluation of the Xpert® MTB/XDR and possibilities of integration into the national diagnostic algorithm – a South African perspective

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BACKGROUND

- In recent years, the global rise and spread of multi-drug resistant (MDR) (resistant to first-line agents rifampicin and isoniazid) and extensively drug resistant (XDR) tuberculosis (MDR with additional resistance to fluoroquinolones (FQ) and second-line injectable agents (SLI)) has disadvantaged control efforts.

- Adequate control of XDR TB requires rapid diagnosis to improve patient outcomes and reducing further transmission.

- In 2018, the WHO had endorsed the Short Course regimen and the Shorter, all-oral, Bedaquiline – containing regimen reducing treatment times to 9-12 months from the standard 18-24 months.

- As both FQ's and SLI's are important components of this regimen – it is critical to report resistance to these drugs as early as possible to ensure adequate patient management.

- Current practice for susceptibility testing requires either phenotypic based methods (the current standard for universal DST) or molecular based methods, if the required infrastructure and skill sets are available.

- Each methodology comes with its own caveat –

    - Phenotypic methods take between 4 – 8 weeks to report
    - Molecular methods despite being faster are restricted to limited targets for inferring drug resistance and performance

- The WHO listed a TB drug-susceptibility test that can detect the most common first and second line drugs as a high-priority with the following product profile;
  - fast
  - low technical skill
  - minimal infrastructure requirement
- In response, Cepheid has developed the Xpert® MTB/XDR assay capable of detecting resistance to ;
  - Isoniazid (low & high)
  - Fluoroquinolones (low & high)
  - Second Line Injectable agents
  - Ethionamide

AIM & OBJECTIVES

AIM - The performance of the Xpert® MTB/XDR* assay was assessed relative to the standard reference methods for MTB detection and drug resistance detection (i.e., phenotypic drug susceptibility assaying (pDST) and sequencing) and to the on-market Xpert® MTB/RIF and Xpert® MTB/RIF Ultra assays.

OBJECTIVES

- To evaluate sensitivity and specificity for drug resistance detection relative to pDST and sequencing independently and as a composite reference standard
- To report the ability of the Xpert® MTB/XDR assay to differentiate between low and high level INH resistance
- To determine Positive Percentage Agreement (PPA) and Negative Percentage Agreement (NPA) of the Xpert® MTB/XDR assay for the detection of MTB relative to the Xpert® MTB/RIF and Xpert® MTB/RIF Ultra assays.

STUDY SITE: Centre for Tuberculosis, National TB Reference Laboratory (WHO TB SRL), NICD/NHLS, South Africa

SAMPLES TYPE: Frozen archived de-identified concentrated sputum specimens pre-characterized phenotypically and genotypically for drugs of interest (samples that have previously been thawed were excluded)

SPECIMEN TESTING PROCEDURES

External Control Testing

- **Three external controls (Plasmids)**
- Run at **Start of the Day**
- **MUT** – 1 mutation/probe
- **WT** – wild-type
- **NEG** – buffer solution

Xpert® MTB/XDR Testing

- Specimens thawed at room temperature
- **"Non-determinate"** result repeat test

Reference Method Susceptibility Testing

- **pDST**
- **Sequencing** of target regions

Mtb Detection Comparison

- **Xpert® MTB/RIF**
- **Xpert® MTB/RIF Ultra**

STATISTICAL ANALYSIS

- Sensitivity and Specificity of the Xpert® MTB/XDR assay for detection of resistance

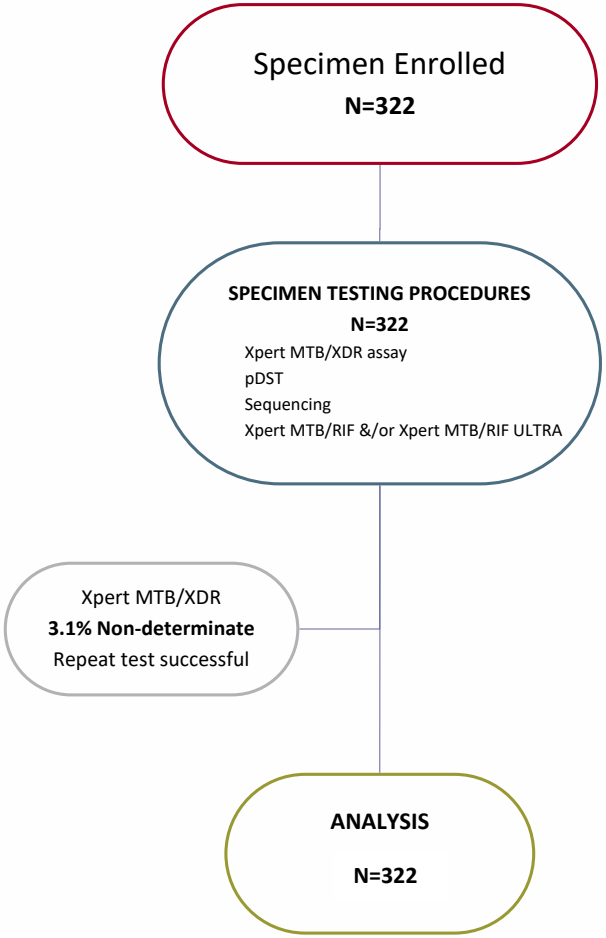
  Acceptance Criteria

Target	*Sensitivity Requirement by Reference Methods*	*Specificity Requirement by Reference Methods*
Isoniazid	≥ 85%	≥ 95%
Flouroquinolone	≥ 85%	≥ 95%
Amikacin	≥ 80%	≥ 95%
Kanamycin	≥ 80%	≥ 95%
Capreomycin	≥ 60%	≥ 95%
Ethionamide	≥ 65%	≥ 95%

- Positive/Negative Percentage Agreement (PPA/NPA) for detection of *Mtb* compared to Xpert® MTB/RIF assays

  Acceptance Criteria – PPA ≥ 95% & ≤ 5.0% non-determinate rate

RESULTS


Specimen Enrolled
N=322
SPECIMEN TESTING PROCEDURES
N=322
Xpert MTB/XDR assay
pDST
Sequencing
Xpert MTB/RIF &/or Xpert MTB/RIF ULTRA
Xpert MTB/XDR
3.1% Non-determinate
Repeat test successful
ANALYSIS
N=322

Performance of Xpert MTB/XDR Test vs. Culture in Smear Negative, Smear Positive, and Overall, for MTB Detection

		Culture				
		Positive			***Negative***	***Total***
		AFB Smear +	***AFB Smear -***	***Overall Culture +***	***Overall Culture -***	
Xpert MTB/XDR Test	***MTB Detected***	220	72	292	0	292
	MTB Not Detected	1	4	5	25	30
	Total	221	76	297	25	322

Performance in ***Culture Positive Smear Positive group***: **Sensitivity: 99.5%** (95% CI: 97.5, 99.9)

Performance in ***Culture Positive Smear Negative group***: **Sensitivity: 94.7%** (95% CI: 87.2, 97.9)

Performance ***Overall***: **Sensitivity: 98.3%** (95% CI: 96.1, 99.3)

Specificity: 100% (95% CI: 86.7.100)

Performance of Xpert MTB/XDR Test vs. Xpert MTB/RIF Ultra Test for MTB Detection

		Xpert MTB/RIF Ultra		
		MTB Detected	***MTB Not Detected***	***Total***
Xpert MTB/XDR	***MTB Detected***	207	0	207
	MTB Not Detected	1	14	15
	Total	208	14	222
	Positive Percentage Agreement	99.5% (95%CI: 97.3-99.9)		
	Negative Percentage Agreement	100.0% (95%CI: 78.5-100.0)		

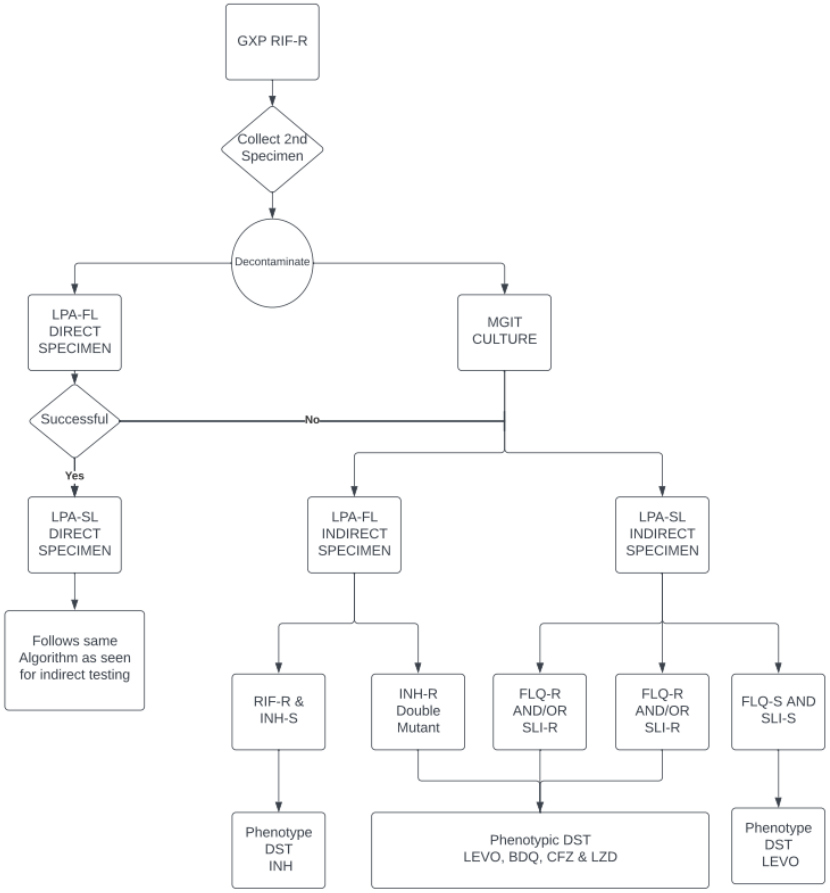
Performance Summary of Sputum Specimens Drug Resistance Detection for Xpert MTB/XDR Test Compared to pDST

Reference	Target	Total	TP	FN	TN	FP	Sensitivity (%)	Specificity (%)
pDST	INH	291	127	13	149	2	**90.7**	**98.7**
	FLQ	231	58	6	167	0	**90.6**	**100**
	AMK	228	50	2	176	0	**96.2**	**100**
	KAN	166	22	4	140	0	**84.6**	**100**
	CAP	167	21	4	142	0	**84.0**	**100**
	ETH	230	75	41	112	2	**64.7**	**98.2**

Performance Summary of Sputum Specimens Drug Resistance Detection for Xpert MTB/XDR Test Compared to Sequencing

Reference	Target	Total	TP	FN	TN	FP	Sensitivity (%)	Specificity (%)
Sequencing	INH	291	128	2	160	1	**98.5**	**99.4**
	FLQ	289	58	3	228	0	**95.1**	**100**
	AMK	286	50	1	235	0	**98.0**	**100**
	KAN	286	51	1	234	0	**98.1**	**100**
	CAP	286	49	1	236	0	**98.0**	**100**
	ETH	292	81	2	209	0	**97.6**	**100**

Current Xpert DR-TB reflex algorithm (South Africa)



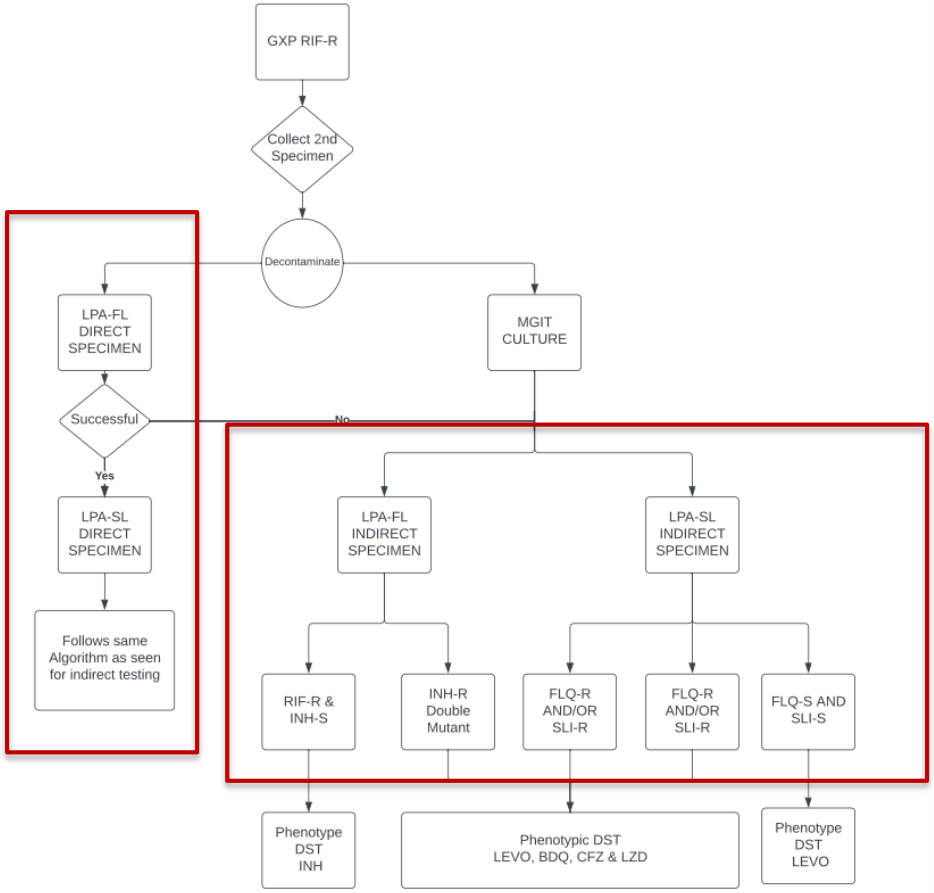
Implementation Strategies

- **STRATEGY 1:** Incorporating Xpert MTB/XDR into the existing DR-TB Reflex as a replacement strategy for LPAfl and LPAsl testing at TB-culture laboratories as an initial implementation phase

- **STRATEGY 2:** Collection of a second specimen for Xpert MTB/XDR testing should Xpert MTB/RIF Ultra detect MTB and rifampicin resistance: Implementation across all Xpert MTB testing laboratories

- **STRATEGY 3:** Processing Xpert MTB/XDR from residual SR-treated Xpert MTB/RIF Ultra as a reflex where MTB has been detected: Implementation across all Xpert MTB testing laboratories – minimum sample volume 2mL or two sputum upfront
  - ***Detect INH-MONO resistance***

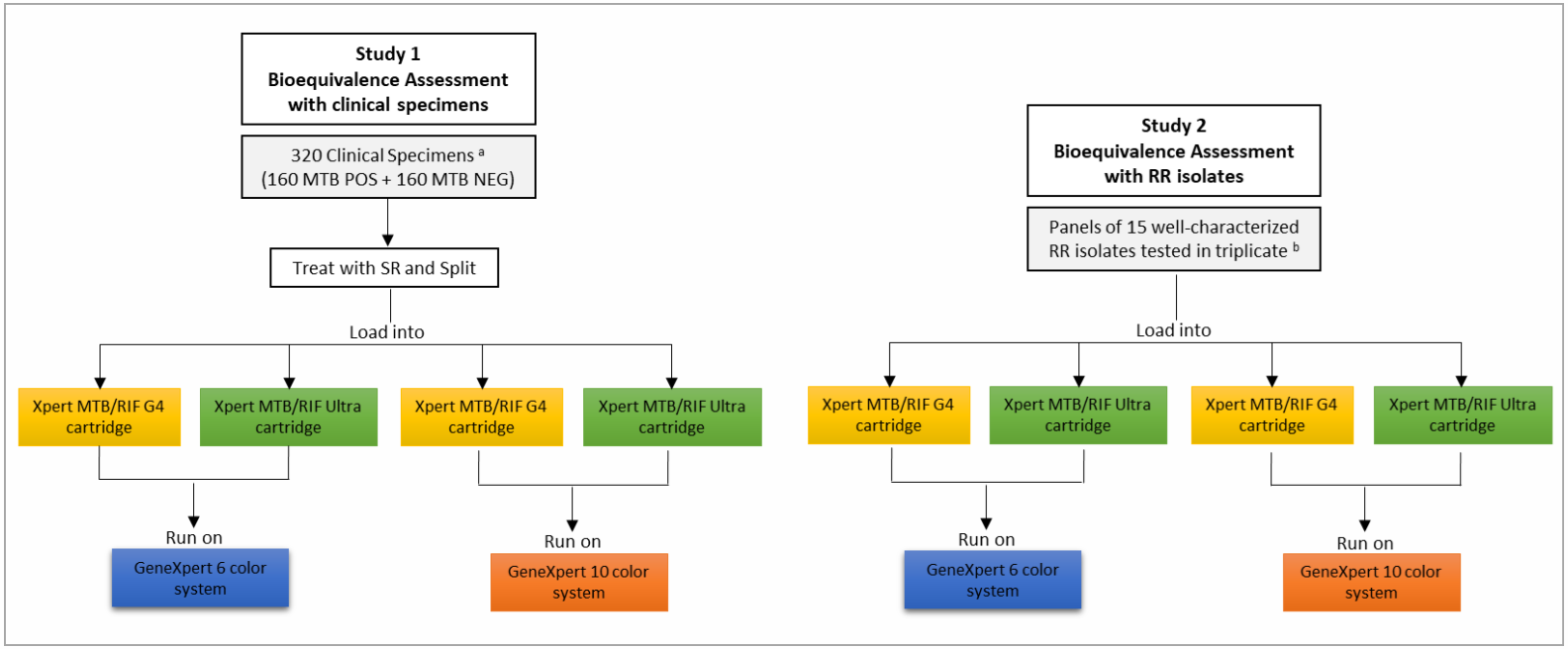
Current Xpert DR-TB reflex algorithm (South Africa)



Infrastructure considerations

Bio-equivalency evaluation of the GeneXpert 6 colour optic channel system and GeneXpert 10 colour optic channel system for TB and RIF resistance detection by the Xpert MTB/RIF G4 and Ultra assays at WHO Supranational Reference Laboratories

Overview of Study 1 & 2 procedures



MTBC detection by Ultra on GXP10 versus GXP6 system

		MTBC Culture positive			MTBC Culture negative		
		GXP10			GXP10		
GXP6	**Ultra result**	***MTB +***	***MTB -***	***Total (%)***	***MTB +***	***MTB -***	***Total (%)***
	MTB +	149	1	**150 (94,3)**	1	0	**1 (0,6)**
	MTB -	3	6	**9 (5,7)**	1	152	**153 (99,4)**
	Total (%)	**152 (95,6)**	**7 (4,4)**	**159 (100)**	**2 (1,3)**	**152 (98,7)**	**154 (100)**

98,4% agreement for MTB detection with a Cohen's k of 0.97

- Discordance mainly related to TRACE results (4/5) and 1 Very Low
  - 3 TRACE detected by GXP 10 only
  - 1 TRACE detected by GXP 6 only
  - 1 Very Low detected by GXP 10 only

Rifampicin resistance detection by Ultra on GXP10 versus GXP6 system

		Rifampicin resistant				Rifampicin susceptible			
		GXP10				GXP10			
	Ultra result	*RR+*	*RR -*	*RI*	*Total (%)*	*RR+*	*RR -*	RI	*Total (%)*
GXP6	*RR +*	53	0	1	54 (100)	0	1	0	**1 (1,25)**
	RR -	0	0	0	0	0	77		**78 (97,5)**
	RI	0	0	0	0	0	1	0	**1 (1,25)**
	Total (%)	**53 (98,1)**	**0**	**1 (1,9)**	**54 (100)**	**0**	**79 (98,75)**	**1 (1,25)**	**80 (100)**

97% agreement for RIF resistance detection with a Cohen's k of 0.94

- Discordance mainly related to Rif Indeterminate calls by both platforms

Conclusions

- The sensitivity and specificity for resistance prediction met the acceptance criteria for all drugs, sensitivities were >90% for all drugs except Ethionamide – due to it being limited to a single target (shared with INH resistance prediction - *inhA promoter*)

- The performance against the pDST reference was marginally lower compared the Sequencing reference. This may be due to pDST being a 'Imperfect Reference' as most of the discordance resolved by sequencing were in agreement with the Xpert® MTB/XDR assay

- The Xpert® MTB/XDR assay was accurate in differentiating resistance levels to Isoniazid – having a direct impact on patient treatment regimen

- Concordance between the Xpert® MTB/XDR assay and Xpert® MTB/RIF Ultra 99.5% - this therefore translates to being able to reflex XDR testing directly off an Xpert® MTB/RIF Ultra resistant sample with a high probability for a successful result

- When testing compatibility for the Xpert ULTRA with both the GXP 6 and GXP 10 color modules on clinical sediments, Xpert MTB/RIF Ultra was highly concordant for MTBC detection, with an overall agreement of 98.4% between the two systems.

- Xpert MTB/RIF and Ultra were also concordant for RIF-resistance detection in clinical specimens, with 97.0% agreement, between GXP6 and GXP10.

- When testing panels of RIF-resistant MTBC isolates, Xpert MTB/RIF Ultra was concordant for RIF-resistance detection, with 97,8% agreement, between GXP6 and GXP10.

- Our findings support manufacturer claims of the GeneXpert 10 color system having backward compatibility with the Xpert MTB/RIF and Ultra assays.

- Future use of this assay, from a “South African” or any TB programme perspective where Xpert®MTB/RIF or Xpert® MTB/RIF Ultra is the primary diagnostic for TB investigation - all RIF resistant specimen are reflexed for further molecular or pDST investigations – the Xpert® MTB/XDR can provide resistance predictions in real-time for up to 6 additional drugs hours apart from the initial Xpert® MTB/RIF or Xpert® MTB/RIF Ultra assay result using the same buffered sample (within 4 hours).

- This would replace two independent molecular tests currently used in our laboratory work-up, improve laboratory turnaround times from days (median 14 days in SA) to hours and enhance appropriate clinical management, particularly the use of Isoniazid and Fluoroquinolones.

- The Xpert® MTB/XDR assay is a fast, robust, sensitive and specific assay for the detection of resistance to Isoniazid, Fluoroquinolones and Second-Line Injectable drugs.

- As the Xpert® MTB/RIF assays have changed the landscape of TB diagnostics, this assay would further contribute to the effective management of drug-resistant TB patients affording health care providers the ability to select the most appropriate treatment regimen rapidly.

THANK YOU

END TB