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# MEMORANDUM

# STANDARDISATION OF PHENOTYPIC DRUG SUSCEPTIBILITY TESTING ACROSS NHLS TB CULTURE LABORATORIES

## Introduction

Molecular testing has largely replaced phenotypic drug susceptibility testing (pDST) for certain core drugs but nonetheless remains important in instances where molecular mechanisms are not clearly defined, insufficiently sensitive for resistance detection, or where levels of resistance are required for dosing purposes (e.g. low/high level moxifloxacin). This memorandum provides guidance on when to perform pDST and which drugs should be tested in light of the updated WHO recommendations and the South African TB diagnostic and treatment algorithms.

## First-line Phenotypic Testing

**Testing the full panel of SIRE drugs is no longer recommended.**

**S: Streptomycin:**

- Streptomycin is no longer included in TB treatment regimens. Testing is of no clinical value, therefore routine testing should be discontinued.

**I: Isoniazid:**

- Although pDST is reliable, the use of MTBDR*plus* has largely replaced pDST, though it misses a proportion of cases with isoniazid resistance. The sensitivity for genotypic resistance detection is estimated at 86% (CI 74–93) Zignol *et al* LID 2018.
- It should be noted that the empiric short course regimen for drug resistant TB uses high dose isoniazid and is included irrespective of the resistance profile.
- However, knowing the phenotypic susceptibility result is useful for clinicians as they manage the isoniazid-associated peripheral neuropathy by reducing the isoniazid drug concentration to a standard dose, if susceptible. The isoniazid-associated peripheral neuropathy may be aggravated by linezolid use in these patients.
- For paediatric cases where isoniazid is susceptible, the injectable agent is dropped. Thus pDST should be performed at 0.1µg/ml for all rifampicin mono-resistant cases.

- Testing may be done on request in patients with rifampicin sensitive TB (Xpert MTB/RIF) without clinical improvement, despite adherence to therapy, and where undetected isoniazid resistance is suspected.
- Isoniazid can be purchased as a single drug from BD and the supplier representative can be contacted for assistance regarding orders and dilution for drug preparation.

**R: Rifampicin:**

- Testing is not required as susceptibility testing is covered by two molecular tests (Xpert MTB/RIF Ultra and GenoType MTBDR*plus*); both with high sensitivity and specificity for rifampicin resistance detection.
- Where discordance between both molecular platforms arises, these should be resolved by *rpoB* gene sequencing rather than pDST.

**E: Ethambutol:**

- Isolates have MICs close to or equal to the critical concentration and thus reproducibility with pDST is problematic.
- The pDST at the current critical concentration correlates poorly with sequencing of the *emb*B gene. Christianson *et al* PlosOne 2014.
- Routine testing for resistance to this drug for patient management is thus not recommended.

**<u>Second line phenotypic DST</u>**

**Fluoroquinolones:**

- The WHO recently released a technical report on critical concentrations for second line pDST. Ofloxacin is not being included in treatment regimens and pDST is thus not recommended for this drug.
- Laboratories should transition to testing specific fluoroquinolones used in current treatment regimens:
    - In South Africa, moxifloxacin is being used in rifampicin-resistant TB treatment regimens.
    - Levofloxacin is part of the WHO recommended short course regimen (SCR) and thus considered for the South African SCR.
    - High dose moxifloxacin is used in treating pre-XDR and XDR-TB cases.
- **The following drugs should be tested as part of the 'DR-TB Reflex' testing algorithm when MTBDR*sl* has detected any resistance:**
    - Moxifloxacin at 0.25 µg/ml – critical concentration
    - Moxifloxacin at 1.0 µg/ml – clinical breakpoint, applies to high dose moxifloxacin i.e. 800mg daily
    - Linezolid[1] at 1.0 µg/ml

[1]Currently being tested only by the Centre for TB/NICD.

However, laboratories can commence testing linezolid once the verification has been completed. A panel of isolates will be distributed to relevant laboratories in August 2018. Laboratories will be contacted by Ms Yasmin Gardee and a Linezolid pDST SOP provided.

**Injectable agents**

- According to the media release on 18/06/2018 by NDOH, all rifampicin-resistant TB patients will now be treated with an injectable free regimen. Thus, there is no longer any clinical value for testing injectable agents routinely (kanamycin and capreomycin).
- Results for the injectable drugs obtained by MTBDR*sl* will still be of use in categorising patients as pre-XDR/XDR to direct further treatment decisions.
- Due to the limited drug options for young children, as the newer drugs have not been fully endorsed for use in this category, amikacin is being used for paediatric DR TB cases and testing should only be performed on request.

**New anti-mycobacterial drugs**

- Bedaquiline will now be included in the treatment regimen for all rifampicin resistant TB patients. Bedaquiline pDST will be implemented to support this.
- The Centre for TB will begin testing bedaquiline and clofazimine on all isolates referred for linezolid testing.
- Testing at designated NHLS laboratories, performing 'DR-TB reflex' is planned for April 2019. A verification panel for this will be sent early in 2019.

**Non-DR-TB reflex requests for DST**

- All specimens sent for diagnostic purposes (not for monitoring) where rifampicin-resistance is identified should follow the standard 'DR-TB reflex' testing and include the pDST panel described above.

**Table: 'DR-TB Reflex' testing – pDST summary**

| Result of MTBDR*plus*/MTBDR*sl* | pDST to be performed |
|---|---|
| Rifampicin Sensitive[2] | None |
| Rifampicin resistance only | Isoniazid 0.1µg/ml |
| MDR | - |
| Pre-XDR/XDR | Moxifloxacin 0.25 µg/ml and 1.0 µg/ml<br>Linezolid 0.1 µg/ml<br>(2019 – Bedaquiline and Clofazimine) |

[2]Trouble shoot discordance and if required perform Xpert MTB/RIF on isolate (or refer for *rpoB* sequencing).

**MEMO Compiled by Centre for Tuberculosis and National Priority Programmes**
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