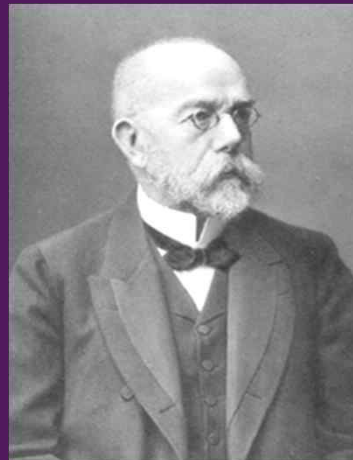


Phenotypic Drug Susceptibility Testing (pDST) for BPaL: MGIT without TBeXIST



This training material is developed by ITRC as technical partner of LIFT-TB

Before start
Prepare storage: MGIT
Prepare storage: LJ
Freezing isolates
Recover isolates

Before you start any kind of experiments, keep in mind

Validate equipment before start

- *If you do not validate equipment, you already failed.*

Don't worry for the new experiment

- *'There is nothing new under the sun'. If you understand background principle, there can be only unfamiliar to you, but nothing entirely new.*

Don't hurry up, but do accurately

- *If I have to take only one between speed and accuracy, I will take accuracy without any hesitation.*

Principle of MGIT DST

MGIT DST Mimics the proportion method

- A known concentration of a test drug is added to one of the tubes
- Growth is compared with the control tube
- If the test drug is active against the strain, it will inhibit the growth.
- There will be suppression of fluorescence, while the GC will be uninhibited and show increasing fluorescence.
- Growth is monitored by the instrument, which automatically interprets results as S or R.

Before
start

Prepare
Drug stock
solution

Media
preparation

Prepare
Inoculum

Inoculation
& setting

Report
results

**Before
start**

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solution

Media
preparation

Prepare
Inoculum

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& setting

Report
results

Recommended CC or concentration ranges of BPaL to determine drug susceptibility for clinical isolates

Purpose: To provide standardized and uniform phenotypic DST procedures for BPaL to LIFT-TB participant countries.

***DST to Bdq and Lzd is performed using critical concentrations (CC). Whereas DST to Pa is performed using minimal inhibitory concentration (MIC)**

CC of B and L is already well established and recommended to adapt for clinical use (*WHO Technical Report on 'critical concentrations for drug susceptibility testing of medicines used in the treatment of drug-resistant tuberculosis, 2018 WHO*). However, **CC for Pa is still on the way to be established and need to monitor MIC levels of each patient.*

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Report
results

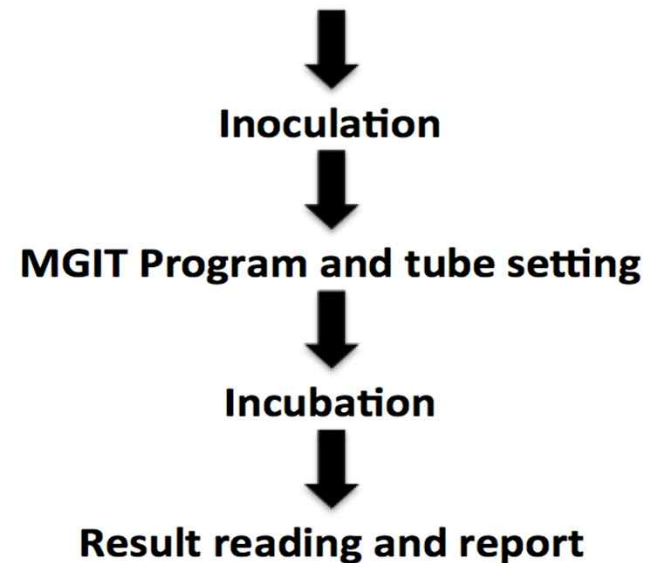
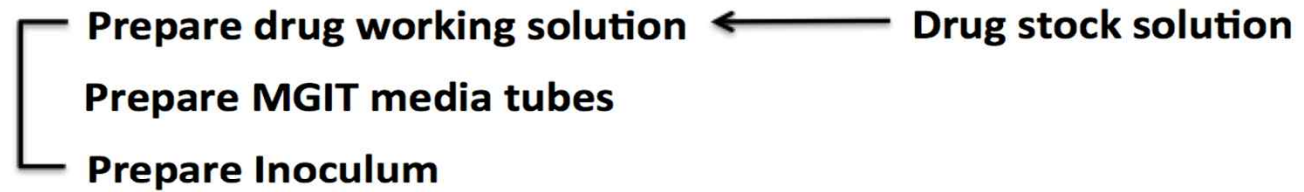
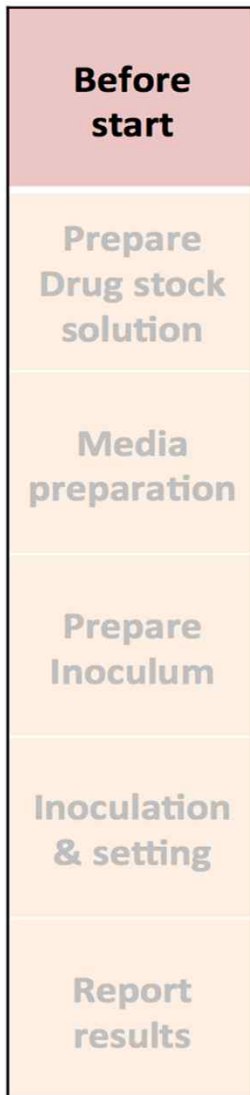
Recommended CC or concentration ranges of BPaL to determine drug susceptibility for clinical isolates

Recommended CC and concentration ranges of BPaL to determine drug susceptibility

Drug	Drug concentration (<u>ug/mL</u>)						
Pa	2	1	0.5	0.25	0.125	0.063	0.031
<u>Lzd</u>	1 (critical concentration)						
<u>Bdq</u>	1 (critical concentration)						

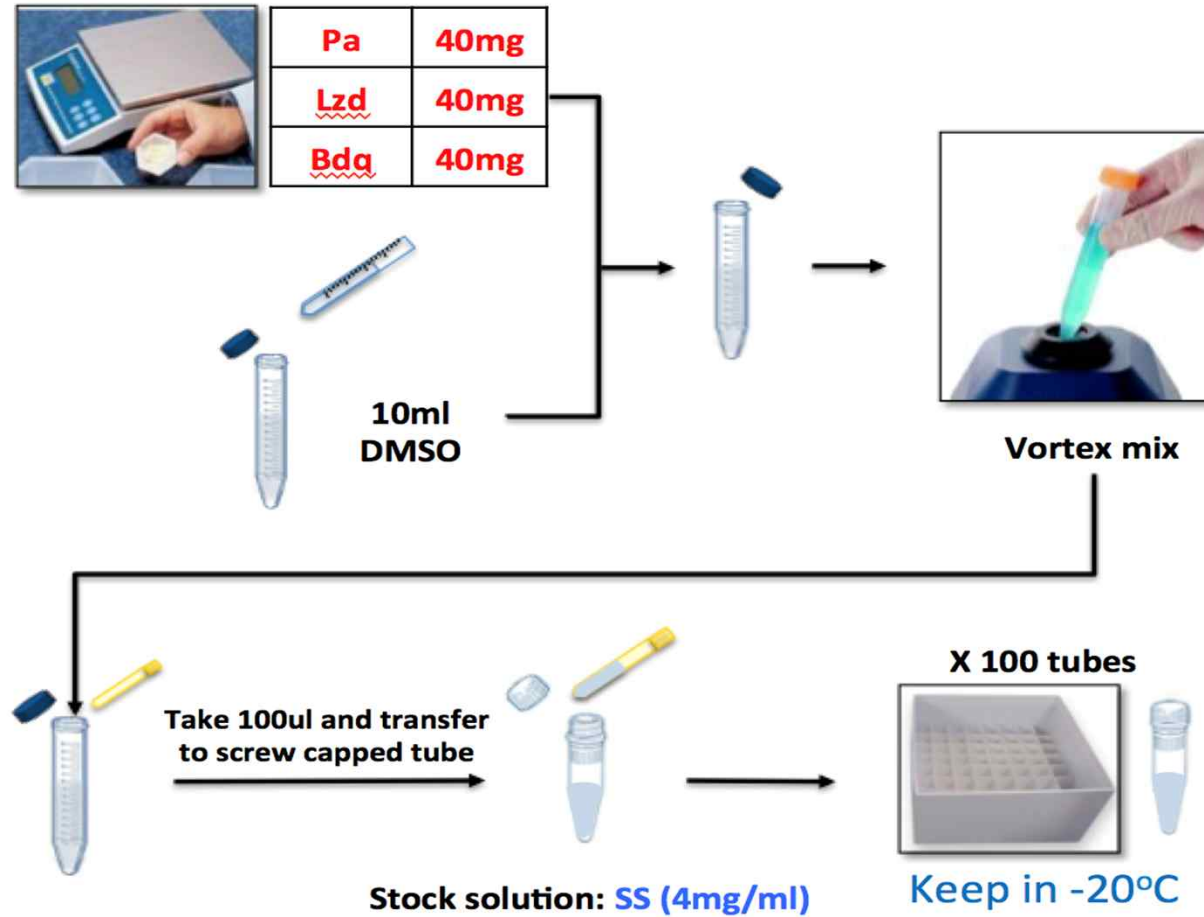
- CC for Bdq and Lzd: Technical Report, 2018 WHO
- Pa: Nix-TB study (Laboratory Manual), TB Alliance technical protocol 'Pretomanid MIC determination using the Bactec MGIT 960 without epicenter/TB eXIST, version 1' and 'Pretomanid MIC determination using the Bactec MGIT 960 with epicenter/TB eXIST, version 2.0'

Overall Procedures

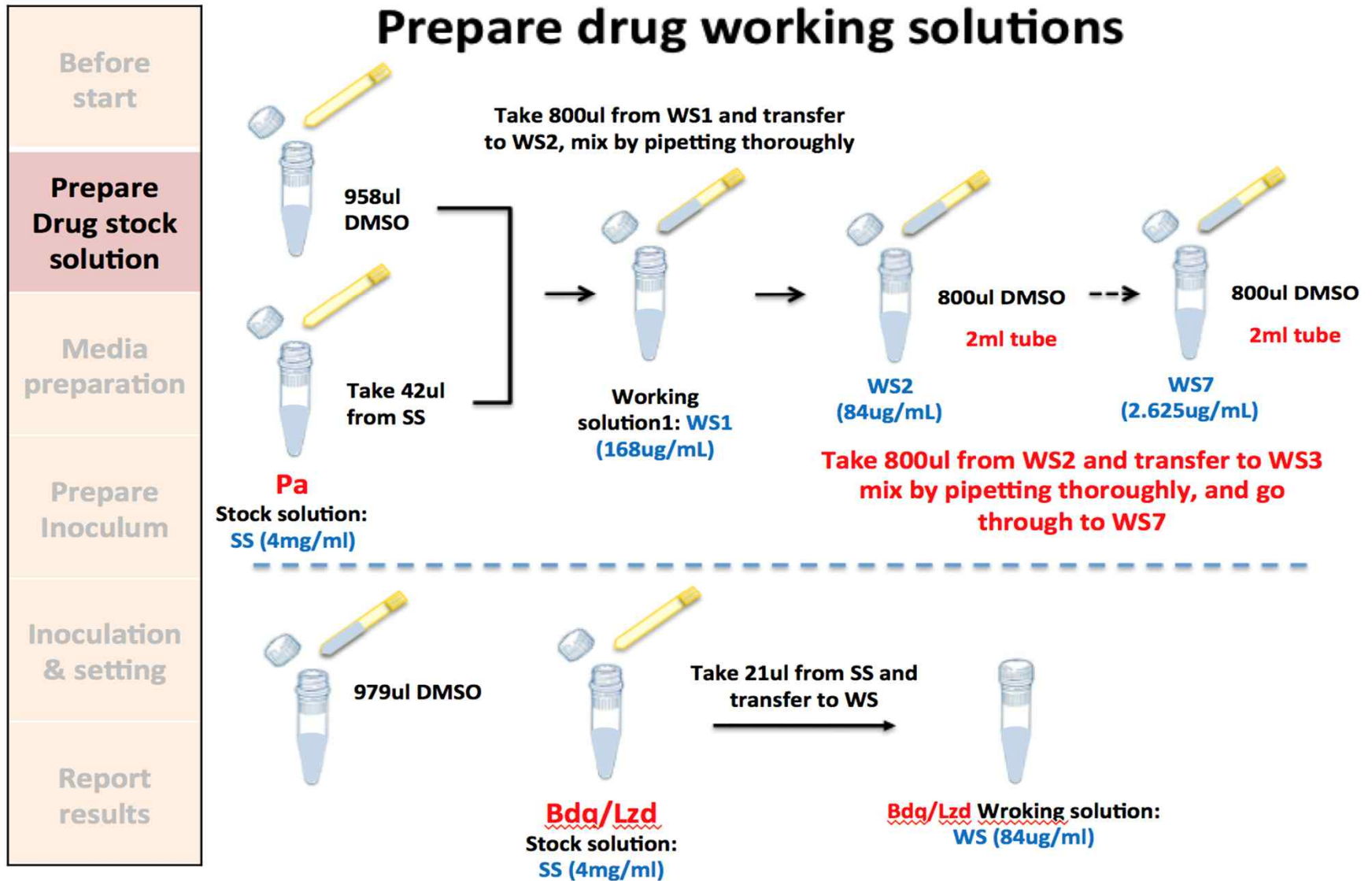


Prepare drug stock solutions and storage

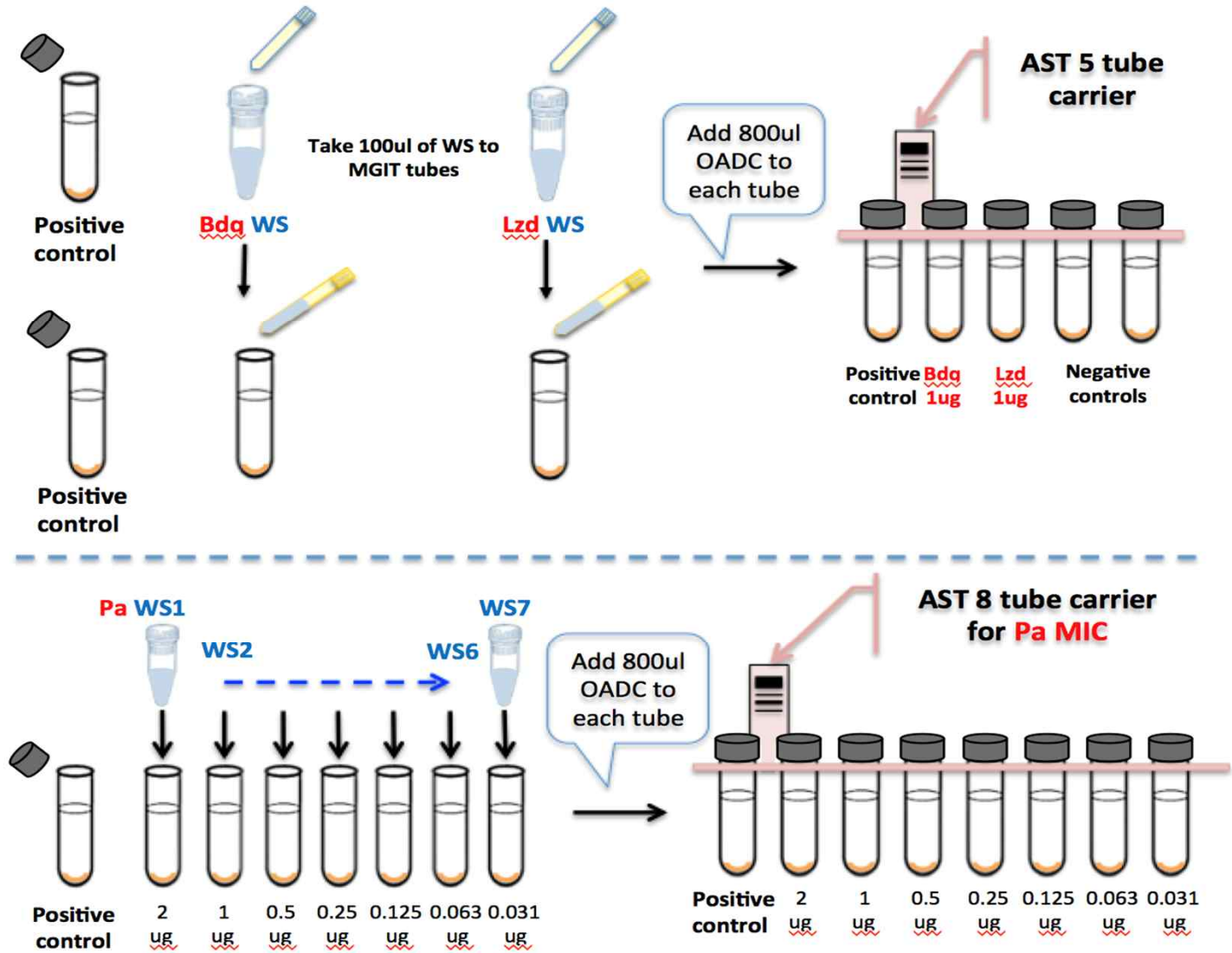
Before start
Prepare Drug stock solution
Media preparation
Prepare Inoculum
Inoculation & setting
Report results



Prepare drug working solutions

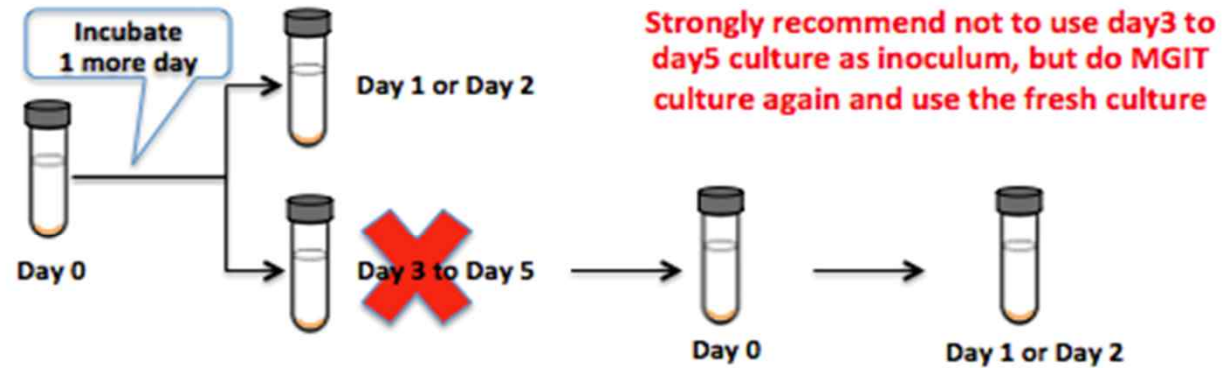


Before start
Prepare Drug stock solution
Media preparation
Prepare Inoculum
Inoculation & setting
Report results

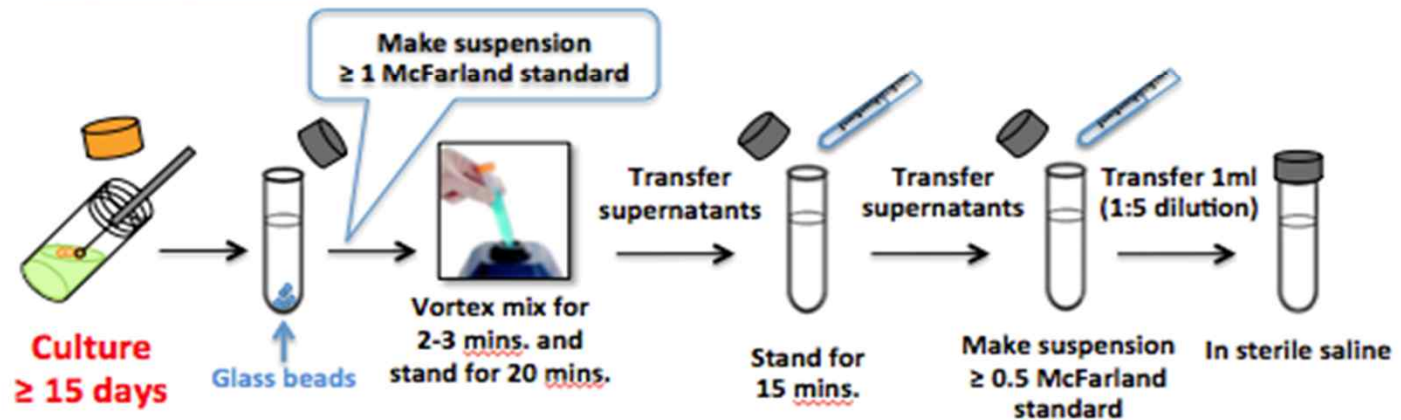


Before start
Prepare Drug stock solution
Media preparation
Prepare Inoculum
Inoculation & setting
Report results

From MGIT culture

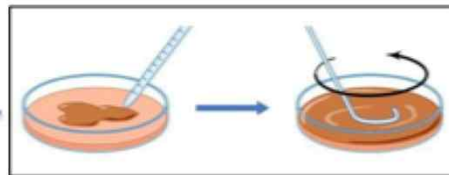
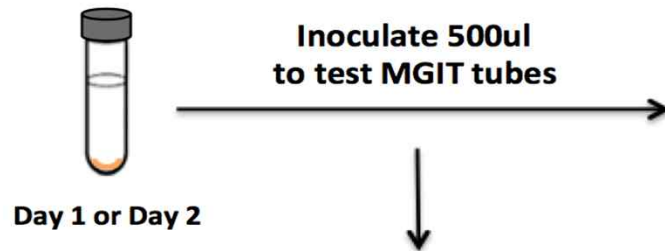


From solid culture

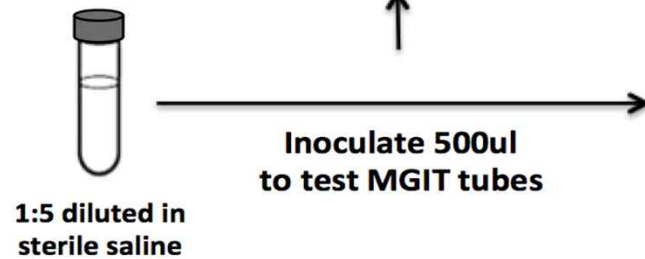


Before start
Prepare Drug stock solution
Media preparation
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Report results

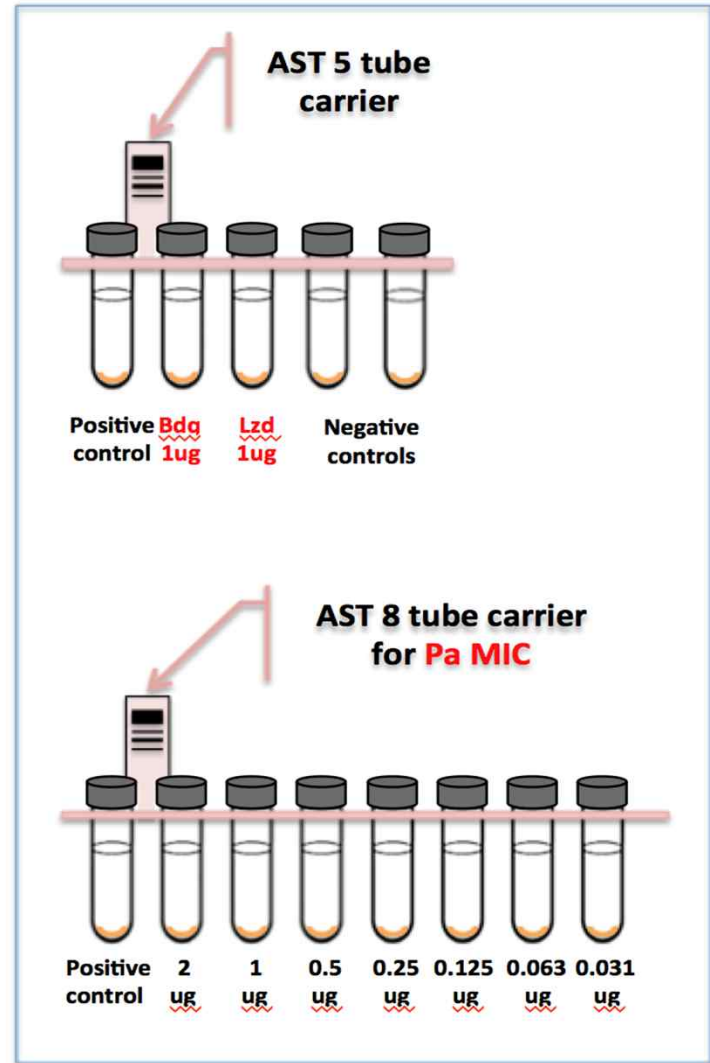
From MGIT culture



Check contamination on blood agar plate: incubate for 2 days



From solid culture



Before start
Prepare Drug stock solution
Media preparation
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Inoculation & setting
Report results

MGIT protocol setting and result interpretation

Use the ‘undefined’ drug protocol, and follow the steps described in the MGIT User’s Manual, DST Instructions

‘Undefined drug’ protocol will end when the GC reaches 400 GU, within 4-13 days. The instrument will signal the AST set is complete; however, only in the ‘undefined drug’ protocol the instrument will not interpret the results-ie., report if culture is resistance or susceptible to th drug. For MIC determinations in the MGIT is important to compare the GU units for each tube/concentration with the others in the set.

All unexpected GU values are indicative of errors in drug dilution preparations or positioning of the tubes in the carrier. In such cases, repeat the test

Reference: TB Alliance technical protocol ‘Pretomanid MIC determination using the Bactec MGIT 960 without epicenter/TB eXIST, version 1’

THANK YOU

PLEASE CONTACT

mlab.itrc@gmail.com

FOR QUESTIONS AND INQUIRIES

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