See Submission and Shipping Instructions on aphl.org/tbdst

Sequencing-Based DST at National PHL DST Reference Center for *Mycobacterium tuberculosis* when pure culture submitted

It is the responsibility of the submitting PHL to report results to the Ordering Clinician and TB Control Program

WGS DST TAT: 10-21 days

Submit Pure Culture on LJ Media or MGIT Broth

Submit sufficient biomass (equivalent to a heaping 1 ul loopful) to ensure timely testing

WGS Genotyping:
The National PHL DST Reference Center for MTB will upload WGS data to the CDC National Surveillance System

Whole Genome Sequencing (WGS) Performed

Phenotypic DST (pDST) Set up

pDST panel that is set up depends on order.
PZA pDST only set up when WGS is U for PZA
See Flow-Charts for pDST for details.

Report WGS DST Results
*If U for PZA, pDST for PZA set up

WGS and pDST = S

WGS = U
pDST = S

WGS = R
pDST = S

WGS = S
pDST = R

WGS = U
pDST = R

WGS = R
pDST = R

Report as Susceptible

Repeat pDST or Report as Indeterminate for PZA

Report as Resistant

Updated 6/10/2024

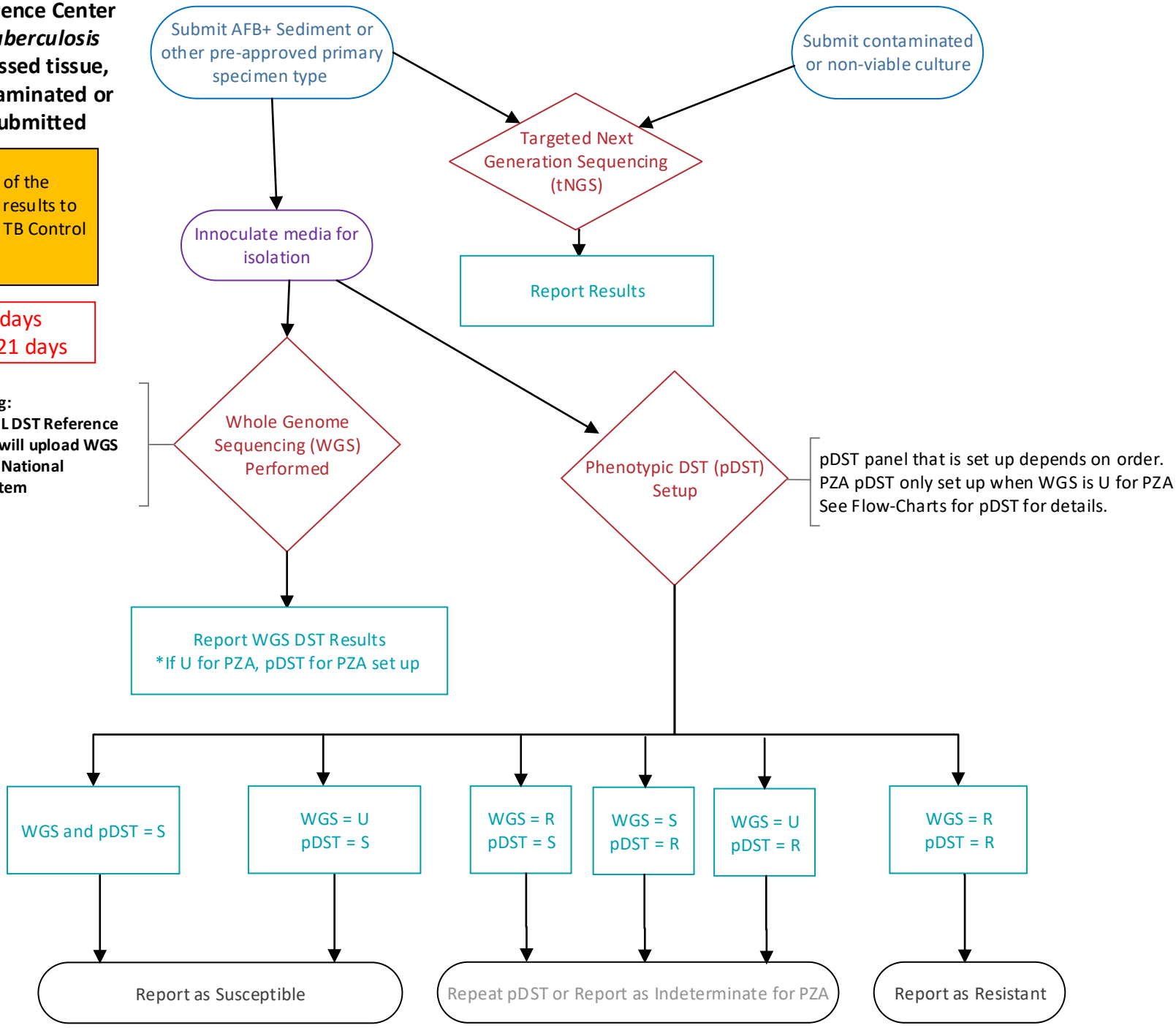
Sequencing-Based DST at National PHL DST Reference Center for *Mycobacterium tuberculosis* when sediment, processed tissue, sterile body fluid, contaminated or non-viable culture submitted

See Submission and Shipping Instructions on aphl.org/tbdst

It is the responsibility of the submitting PHL to report results to the Ordering Clinician and TB Control Program

tNGS TAT: 7-10 days
WGS DST TAT: 10-21 days

WGS Genotyping:
The National PHL DST Reference Center for MTB will upload WGS data to the CDC National Surveillance System



Updated 6/10/2024

First-Line DST (FL-DST) at National PHL DST Reference Center for *Mycobacterium Tuberculosis*

It is the responsibility of the submitting PHL to report results to the Ordering Clinician and TB Control Program

Submit pure culture on LJ Media or MGIT Broth
-OR-
Specimen Reflexed from tNGS

Submitting PHL should keep 1 mL aliquot and submit remainder to MDL
Isolates on Solid Media Acceptable

WGS-DST Performed and Results Reported (If not already)

Assessed for Concordance as Shown pg 1 and Below

First-Line DST Performed when ordered

First Line Panel*
Rifampin (RIF), 1 µg/mL
Isoniazid (INH), 0.1 µg/mL^
Ethambutol (EMB), 5 µg/mL
Purity plate also setup to check for contamination or NTM
*pDST for PZA is performed only if "Uncertain" WGS results are obtained.
^INH tested at 0.4 µg/mL when INH resistance detected by any method

No Resistance Detected (including genotypic data)

MDL Reports Results to Submitter

Resistance Detected to at least one first-line drug

If RIF-R is detected, the sample will be referred to CDC for MDDR
PZA mono-resistance reflexed to SL-DST by request only

Smear or purity plate checked to rule out contamination

No Contamination Detected
Phenotypic and Genotypic Results Concordant

Simultaneously

MDL Reports Preliminary Results to Submitter

SL-DST Performed

MDL Reports Results to Submitter

No Contamination Detected
Phenotypic and Genotypic Results Discrepant -OR- No genotypic data available

MDL Reports Preliminary Results to Submitter, See pg 1 for Details

AND/OR

Repeat FL-DST

tNGS For Confirmation

MDL Reports Results to Submitter
*tNGS reported only if it confirms MGIT DST Result

Contamination Detected

Simultaneously

tNGS For Confirmation

Request Pure Culture for Repeat

MDL Reports Results to Submitter

If no mutation was detected or amplification fails results will be reported.

No further action will be taken pending receipt of pure culture

Updated 6/10/2024

DST for the 4-month regimen at National PHL DST Reference Center for *Mycobacterium Tuberculosis*

Submit pure culture on LJ Media or MGIT Broth
-OR-
Specimen Reflexed from tNGS

Submitting PHL should keep 1 mL aliquot and submit remainder to MDL
Isolates on Solid Media Acceptable

It is the responsibility of the submitting PHL to report results to the Ordering Clinician and TB Control Program

WGS-DST Performed and Results Reported (If not already)

Assessed for Concordance as Shown pg 1 and Below

Alternative First-Line DST Performed

MIGT Panel*
Rifampin (RIF), 1 µg/mL
Isoniazid (INH), 0.1 µg/mL^
Moxifloxacin (MOX), 0.25 µg/mL
Purity plate also setup to check for contamination or NTM
*pDST for PZA is set up only if "Uncertain" WGS results are obtained.
^INH tested at 0.4 µg/mL when INH resistance detected by any method

No Resistance Detected
(molecular and first line testing reported as results become available)

MDL Reports Results to Submitter

Resistance Detected to RIF, INH or MOX

SL-DST will include EMB instead of MOX
If RIF-R is detected, the sample will be referred to CDC for MDDR
PZA mono-resistance reflexed to SL-DST by request only

Smear or purity plate checked to rule out contamination

No Contamination Detected
Phenotypic and Genotypic Results Concordant

Simultaneously

MDL Reports Preliminary Results to Submitter

SL-DST Performed

MDL Reports Results to Submitter

No Contamination Detected
Phenotypic and Genotypic Results Discrepant -OR- No genotypic data available

MDL Reports Preliminary Results to Submitter, See pg 1 for Details

AND/OR

Repeat FL-DST

tNGS For Confirmation

MDL Reports Results to Submitter
*tNGS reported only if it confirms MGIT DST Result

Contamination Detected

Simultaneously

tNGS For Confirmation

Request Pure Culture for Repeat

MDL Reports Results to Submitter

If no mutation was detected or amplification fails results will be reported.

No further action will be taken pending receipt of pure culture

Updated 6/10/2024

Second-Line DST (SL-DST) at National PHL DST Reference Center for *Mycobacterium Tuberculosis*

It is the responsibility of the submitting PHL to report results to the Ordering Clinician and TB Control Program

Testing Criteria
1. Resistance to any first-line drug is identified by molecular DST or growth-based DST
2. SL-DST requested due to risk factors or clinical need

If RIF-R is detected, the sample will be referred to CDC for MDDR
PZA mono-resistant isolates will not be automatically reflexed to SL-DST

WGS-DST Performed and Results Reported (If not already)

Assessed for Concordance as Shown pg 1 and Below

Second-Line DST Performed

Second Line Panel
Amikacin (AMK), 1.5 µg/mL
Capreomycin (CAP), 3 µg/mL
Ethionamide (ETA), 5 µg/mL
Kanamycin (KAN), 3.5 µg/mL
Rifabutin (RFB), 0.5 µg/mL
Moxifloxacin (MOX), 0.25 µg/mL

No Resistance Detected (phenotypic and genotypic results concordant)

MDL Reports Results to Submitter

Resistance Detected to MOX (phenotypic and genotypic results concordant)

Simultaneously

MDL Reports Preliminary Results to Submitter

MOX MIC at 0.5, 1, 2, 3µg/mL Upon Request

MDL Reports Results to Submitter

Resistance Detected to AMK, CAP, ETA, KAN, RFB (phenotypic and genotypic results concordant)

MDL Reports Results to Submitter

Phenotypic and genotypic results discordant

MDL Reports Preliminary Results to Submitter, see pg 1 for details

Repeat SL-DST

AND/OR

tNGS For Confirmation

MDL Reports Results to Submitter
*tNGS reported only if it confirms MGIT DST Result

Updated 6/10/2024