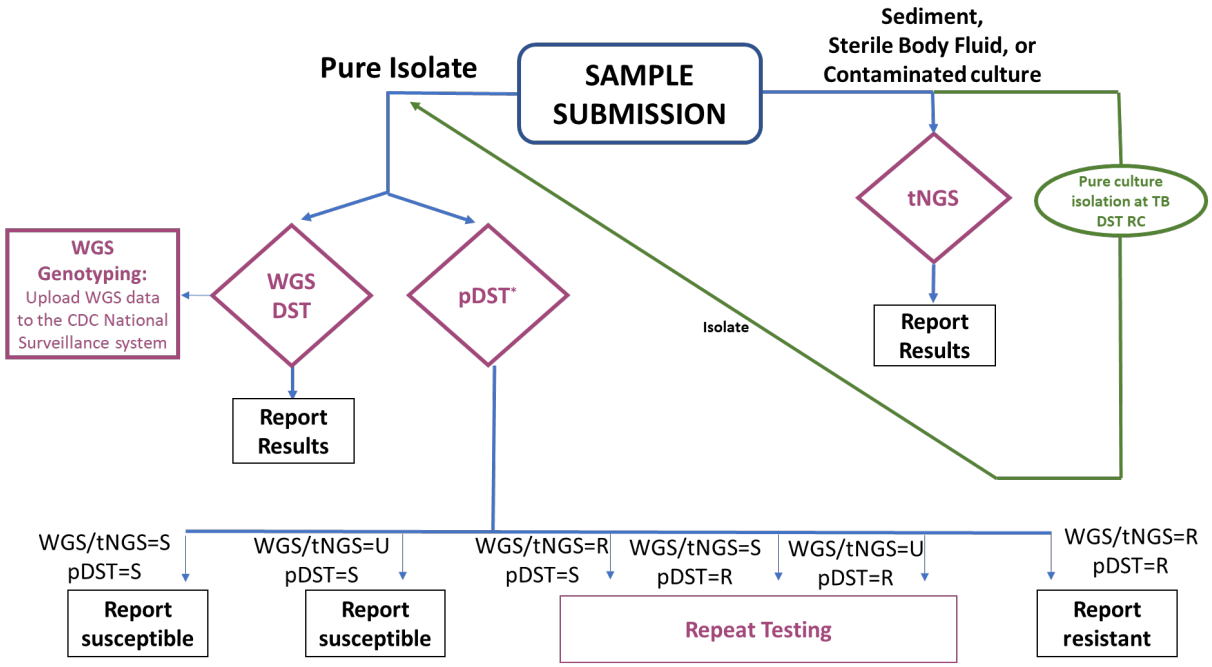
TB Sequencing-based Drug Susceptibility Testing FAQs: Whole Genome Sequencing and targeted Next Generation Sequencing

Q: What is the drug susceptibility testing algorithm at the TB Drug Susceptibility Testing Reference Center for sequencing-based and phenotypic methods?

A: The TB Drug Susceptibility Testing (DST) Reference Center (TB DST RC) relies on sequencing-based DST (sbDST) using whole genome sequencing (WGS) and targeted Next Generation Sequencing (tNGS) to provide comprehensive predictions of drug susceptibility/resistance in MTBC. Phenotypic DST (pDST) is performed using the Bactec MGIT system and remains an important reference method, though its turnround time is generally longer than the sbDST methods.

At the TB DST RC, sbDST and pDST are performed in parallel (with the exception of PZA). Results of sbDST and pDST are reported independently as those become available, however, both are taken into consideration in order to address discrepancies. WGS-DST is the primary method when pure cultures are submitted, while tNGS-DST can be performed directly on processed specimens and mixed/nonviable cultures. WGS data generated for DST purposes can also be used for TB genotyping; WGS data generated at TB DST RC is shared with the CDC's national TB surveillance system (unless submitter opts out). For submitted sediments, TB DST RC will attempt isolation of pure culture and, if successful, WGS-DST will be performed, and results will be reported (in addition to tNGS performed directly on sediment). Below is the high-level overview of the testing workflow at TB DST RC:



*In case of discrepancies between sequencing-based DST and pDST, results will be investigated for individual loci and drugs. PZA testing has a different algorithm.

Q: What is the principle of the sequencing-based drug susceptibility testing methods used by the TB Drug Susceptibility Testing Reference Center?

A: The TB DST RC utilizes two different methods for sequencing-based DST (sbDST): whole genome sequencing (WGS) and targeted Next Generation Sequencing (tNGS). The WGS-DST assay leverages short-read Illumina next generation sequencing technology to sequence the entire genome of the microorganism. The tNGS-DST assay similarly utilizes Illumina sequencing technology, however unlike WGS, MTBC genome loci associated with resistance are amplified prior to sequencing using Deeplex® Myc-TB kit (GenoScreen, Lille, France) and custom primers, which allows detection of low amounts of MTBC DNA directly from processed clinical specimens.

Subsequent analysis with an in-house developed bioinformatics pipeline is used to detect mutations and generate predicted drug interpretations for both WGS and tNGS methods. This information is critical for the effective treatment of patients suffering from tuberculosis (TB) and initiation of public health interventions.

Both WGS and tNGS assays also allow MTBC species confirmation, while generated WGS data can also be used for MTBC species confirmation and genotyping.

Q: I do not see WGS-DST or tNGS-DST assays on the TB DST RC Lab Web Portal. How do I order either of those test options?

A: When the "Sequencing-based DST" option is selected on the TB DST RC Lab Web Portal, either the WGS or tNGS assay for drug susceptibility prediction will be automatically assigned depending on the submitted material indicated by the submitter on test requisition form:

- If a pure culture is submitted, WGS-DST will automatically be ordered.
- If a sediment, sterile body fluid, processed tissue, or mixed/non-viable culture are submitted, tNGS-DST will automatically be ordered.

The submitter does not need to specifically select tNGS or WGS.

Q: Can I still order pyrosequencing for cultures?

A: Submitters can order sequencing-based DST on all acceptable specimen types, but they are not able to choose the sequencing method as this is determined by the type of submitted material. If a *sediment, sterile body fluid, processed tissue,* or *mixed/nonviable culture* is submitted, tNGS will be performed in place of PSQ starting June 10th, 2024 as the primary molecular DST method. WGS will be performed on submitted isolates.

While the turnaround time for sequencing-based DST is longer than for PSQ, this method provides much more comprehensive molecular resistance detection than PSQ; WGS additionally allows determination of genotype.

Q: Will WGS-DST be performed on all isolates submitted for phenotypic DST regardless of if I request it on submittal form?

A: Yes. When requesting DST in the TB DST RC Lab Web portal, the "Sequencing-based DST" option is selected as a default test and submitters cannot opt-out. When a pure culture is submitted, this means that we will perform both pDST and WGS-DST and will issue results for the corresponding tests as separate reports. This will not delay phenotypic testing since these tests are set up and reported independently, without reflex. The exception is for PZA pDST which would be performed only after applicable WGS results are obtained.

Q: Will phenotypic DST be performed on all isolates submitted for WGS-DST regardless of whether I request it on submittal form?

A: No, a submitter should request pDST specifically. The submitter must ensure that the test requisition is correct to obtain timely pDST reports. We recommend that pDST (first-line **or** the alternative 4-month regimen first-line drug panel) is always requested in addition to sequencing-based DST, unless pDST has already been performed.
In specific circumstances, pDST reflex testing may be performed to confirm WGS results even if not requested by the submitter, but this will be done on a case-by-case basis.

Q: Is there still a utility in ordering phenotypic DST if I'm already getting WGS-DST for my isolate?

A: Yes. The phenotypic and WGS results are complementary. In particular, there may be cases for which a mutation is not detected in genes associated with resistance, but the isolate is phenotypically resistant due to an unknown mechanism. Phenotypic DST is also important to clarify an effect of the mutations of uncertain significance detected by WGS. Hence, if phenotypic DST has NOT been performed already at a clinical or public health laboratory, we recommend ordering first-line or the alternative 4-month regimen first-line drug panel for pDST, in parallel with WGS-DST. Please note that for some drugs (e.g. PZA), the WGS results may be more reliable than those of the phenotypic assay.
In the future, the TB-DST RC will evaluate modifying the testing algorithm to utilize WGS-DST as the primary clinical test for first line drug resistance determination. In such a workflow, samples would only be reflexed to pDST for the confirmation of resistance-conferring mutations, when mutations with an uncertain effect are detected, and upon submitter request. Submitters will be notified in the event of any service changes.

Q: What is the typical time frame for phenotypic versus sequencing-based DST if both tests are ordered? If the phenotypic result becomes available later, does that require physicians to revisit both sequencing-based and phenotypic results?

A: Sequencing-based DST results will normally be available and reported to the submitters before phenotypic results. Expected turnaround times are:

- tNGS TAT 7-10 days
- WGS TAT 10-21 days
- phenotypic DST TAT 19-45 days

We recommend reviewing and evaluating all molecular and phenotypic DST results, in addition to other clinical and laboratory data as results become available. Discrepancies between sbDST and phenotypic results are possible and consultation is available by contacting TB DST RC (Matthew.Sylvester@cdph.ca.gov, Varvara.Kozyreva@cdph.ca.gov, CDPHTBDST@cdph.ca.gov), your state TB program (https://www.cdc.gov/tb/php/tb-programs/index.html), and TB Centers of Excellence (TB COE) (877-390-6682; https://www.cdc.gov/tb/education/tb_coe/default.htm)

Q: Should I expect WGS-DST results for a submitted sediment if I've already received a tNGS report?

A: Yes, the TB DST RC will attempt culture isolation from processed primary specimens (sediments and tissues), as well as sterile body fluids, and if successful, WGS will be performed. WGS-DST results will be reported to the submitter, and raw WGS data will be uploaded for national surveillance (unless submitter opted out). If the TB DST RC is unable to isolate a pure culture, an isolate will be requested from the submitter. Culture isolation will NOT be attempted for submitted mixed or nonviable cultures.

Q: What is the difference between the "Sequencing-based DST" and "WGS Genotyping" test options on TB DST RC Lab Web Portal? Which one should I pick for my isolate if I would like to get WGS-based resistance prediction??

A: The "Sequencing-based DST" test option is selected by default in the TB DST RC Lab Web Portal. If submitted material is a pure culture, WGS-based DST will be performed for your isolate.
WGS genotyping implies WGS for the purposes of surveillance (including upload to CDC for national-level surveillance) and outbreak detection. Note that on the Lab Web Portal, when ordering "Sequencing-based DST", "WGS Genotyping" is pre-selected when the material type is "pure culture", as the same WGS procedure is used to generate data for WGS-DST and for WGS genotyping analysis. The submitter can opt out of WGS genotyping, which would mean that TB DST RC will not submit WGS sequences to the CDC, and the submitting lab will be responsible for ensuring that WGS is performed and data is submitted to the CDC. We recommend leaving the WGS genotyping option selected when ordering WGS-DST unless an isolate from the patient has already been sent for national WGS surveillance genotyping elsewhere.

Q: If I request WGS-genotyping, should I expect a report with genotyping results from the TB DST RC?

A: A WGS-genotyping report will be issued to the submitter only when TB DST RC is unable to perform WGS genotyping (e.g. due to culture appearing mixed upon sequencing) and will include a request for the submitter to send a pure isolate.
When WGS is performed, (i.e.: for either submitted pure cultures or cultures isolated from processed specimens by the TB DST RC), WGS-genotyping data will be uploaded to the CDC for national surveillance. All genotyping results will be available in TB GIMS.

Q: If a culture is determined to be mixed by WGS, will it automatically be reflexed for tNGS?

A: Yes, cultures for which WGS results indicate contamination will be reflexed to tNGS automatically. However, culture isolation for pDST and WGS will not be performed on mixed and non-viable cultures and submitters will be requested to send a pure isolate for pDST or genotyping purposes.

Q: Our lab performs phenotypic DST in-house. Should we send all new TB isolates to TB DST RC for WGS DST since it covers so many different AMR genes, or only submit those isolates that we have problems with from our culture-based DST?

A: As TB DST RC will be submitting WGS data for national TB surveillance, it would be ideal to submit all new TB isolates to TB DST RC for WGS DST. Sequences generated for DST will be also used for TB genotyping purposes.

Q: What is the difference between TB DST RC TB WGS-DST and CDC's MDDR service for molecular DST?

A: The main differences are:

1. Testing method/Acceptable sample types: The CDC's MDDR service is a tNGS assay that can be performed on primary sediments and cultures, while TB DST RC performs WGS-DST when pure cultures are submitted and tNGS-DST for processed specimens.
2. Covered gene loci: The TB WGS-DST test provided by TB DST RC will cover all targets available from CDC's MDDR service and includes some additional loci. TB DST RC's tNGS-DST assay includes additional loci for Ethionamide and Capreomycin but is limited to *Rv0678* locus for Bedaquiline and Clofazimine. The exact genetic coordinates of the regions investigated by the CDC is available on MDDR website and TB DST RC's WGS and tNGS assay reportable genomic regions are shown below.

Q: If I sent an isolate to the TB DST RC for WGS-DST or WGS-genotyping, do I still need to submit the isolate to the Michigan State Public Health Laboratory (Genotyping Lab)?

A: No, submission of TB isolates to TB DST RC for WGS genotyping will replace the need to send to MI.

Q: Do I need to send an Isolate Submission Form (ISF) with patient metadata to the TB DST RC when requesting TB WGS genotyping (like what is done for the Michigan Genotyping lab submissions)?

A: No, all patient data for TB GIMS uploads will be collected via TB DST RC Lab Web Portal (ETOR).

Q: Are we still required to send samples with known RIF-resistance to the CDC MDDR?

A: Submission of isolates and sediments with known RIF-resistance to CDC MDDR is no longer required; all isolates can be submitted to the TB DST RC. Samples confirmed as RIF-R or MDR by the DST RC will be referred to the CDC.

Q: Is false-resistance possible in either the WGS DST or the tNGS-DST assay if MTBC culture is contaminated with nontuberculosis mycobacteria?

A: In our validation study, we saw no evidence of this being a concern. We evaluated the specificity of both WGS-DST and tNGS-DST by analyzing sequences of various NTM species with our bioinformatics pipeline for resistance prediction, and for those samples the pipeline did not return any results that could be mistaken for MTBC. Additionally, we performed an *in silico* contamination study where we mixed MTBC and NTM DNA in different proportions and also did not observe any interference with WGS-DST or tNGS-DST results from NTM contamination that would result in false-positive or false-negative resistance detection. However for WGS-DST, above a certain percentage, contamination resulted in assay failure. Therefore, even though the WGS DST assay is tolerant to some level of contamination, we still require a pure culture. The tNGS-DST assay is better suited for detection of TB resistance targets from mixed cultures.

Q: Can the WGS or the tNGS assay provide identification for all MTBC species?

A: Even though WGS technology has the potential to identify all MTBC species, the WGS DST assay we validated only provides identification for *M. tuberculosis* and *M. bovis* species, and further differentiates the BCG strain of *M. bovis*. In cases when DNA of MTBC organism is detected but cannot be identified as either *M. tuberculosis* or *M. bovis*, it will be reported as "DNA of *Mycobacterium tuberculosis* complex detected". If no DNA of MTBC is detected, this will be also reflected on the report.
The tNGS method has been validated for MTBC ID confirmation as well, but unlike WGS, tNGS only allows confirmation of MTBC DNA presence and differentiation of *M. bovis* from non-*M. bovis* MTBC. *M. tuberculosis* species or BCG strain of *M. bovis* are not specifically differentiated by tNGS. Please note that the tNGS assay is not intended for diagnosis of TB disease and negative results for the detection of MTBC DNA in the specimen do not rule out presence of TB.

Q: What is the limit of detection for the tNGS assay? What AFB grade or Gene Xpert MTB/RIF Ct values correlate with successful sequencing by tNGS?

A: Based on our validation study, the MTBC tNGS-DST assay is expected to perform well with processed clinical specimens demonstrating a Xpert MTB/RIF PCR Ct value less than or equal to 28, or approximately AFB 1+ microscopic grading; samples with lower TB loads may or may not sequence well. Pre-approval is required for specimens with rare or no AFB observed, or with a Xpert MTB/RIF Ct value higher than 28 since the probability of successful sequencing is lower.

Q: In the case of heteroresistance, what is the minimum percent of the resistant strain subpopulation that can be detected by WGS or tNGS DST assay?

A: Due to a variety of factors including sampling bias, a possible shift in the representation of the resistant subpopulation during culture growth *in vitro* for WGS or due to bias introduced by amplification in case of tNGS, it is difficult to correlate the exact percentage of the resistant subpopulation identified during testing to what exists in the patient. However, in our validation study we determined that the limit of detection (LOD) for the mutation allele frequency in TB genome that ensures high accuracy of the WGS-DST assay is around 10%. tNGS-DST is comparable to WGS, but due to the difference in amplification efficiency of different loci, it has an additional variability in detected allele frequency. Both sequencing-based DST assays are well-suited for detection of heteroresistance in MTBC but cannot be used quantitatively.

Q: How should I interpret the results of WGS-DST or tNGS-DST report?

A: The most important information on the WGS-DST or tNGS-DST report for clinical use is the sequencing-based DST interpretations that are provided on the report opposite the corresponding drug name. The user may review information about detected mutations for each individual gene displayed on the report; however, all detected genomic variations and their potential effects are summarized for each drug based on the validated by TB DST RC interpretation algorithm. Below are the possible sequencing-based DST result options for individual drugs and their meaning:

Drug interpretation result as it appears on clinical report	**Explanation**
Mutation(s) associated with resistance to XXX detected	There is strong evidence of association of the detected mutation with phenotypic resistance. Susceptibility is highly unlikely. This prediction is based on the data collected by WHO and the global TB community on correlation of specific mutations with resistance in phenotypic assays, and overall scientific knowledge of resistance mechanisms in MTBC.
The detected mutation(s) have uncertain significance. Resistance to XXX cannot be ruled out	Insufficient amount of evidence is available regarding the association of the detected mutation (s) with resistance. Confirmatory pDST is needed for definitive resistance determination.
No mutations associated with resistance to XXX detected	Resistance is unlikely, but cannot be ruled out due to: - Mutations in loci not covered by WGS or tNGS that contribute to unknown mechanisms of resistance; or, - Heteroresistance below the limit of detection (LOD) of the sequencing-based assay
Predicted resistance to rifampin OR Predicted susceptibility to rifampin	There is a high likelihood that the strain is resistant/susceptible to rifampin. Resistance/susceptibility to rifampin can be predicted with higher certainty than other drugs, and this is reflected in the reporting language.
Predicted susceptibility to rifampin. The detected synonymous mutation(s) do not confer resistance	Synonymous mutations are normally not reported for drugs other than rifampin, however, synonymous mutations in *rpoB* are to resolve potential discrepancies with PCR-based assays, e.g., silent mutations that cause false-positive results in the Xpert MTB-RIF PCR assay.
Predicted low-level resistance to rifampin. May test susceptible by phenotypic methods	The detected mutation is known to cause low-level, yet clinically relevant, resistance to rifampin. When strains harboring such mutations are tested by pDST at a critical

	concentration of 1 ug/mL, they may test susceptible.
Pending Retest	One or more gene targets had insufficient coverage, which does not allow confident susceptibility predictions for the corresponding drug. This is a preliminary report, and the sample will be re-sequenced.
Not all targets could be sequenced; resistance to XXX cannot be ruled out	One or more gene targets had insufficient coverage, which does not allow confident susceptibility predictions for the corresponding drug. Sequencing has been repeated but successful sequence could not be obtained. This is a final report. Please contact the lab to see if sample resubmission is necessary.

Q: For rifampin, why does the WGS/tNGS-based predictions indicate "Predicted susceptibility" or "Predicted resistance", but for other drugs it only states that mutation(s) associated with resistance are detected/not detected?

A: We have more certainty in WGS/tNGS predictions for rifampin because we have much more extensive data on the effect of *rpoB* mutations on RIF resistance and mutations in the hot-spot (rifampin resistance determining region) of the *rpoB* gene are known to be a predominant mechanism of RIF resistance in clinical strains. Consequently, we give a more definitive statement about predicted resistance or susceptibility. We also have more knowledge of correlation of different mutations with different levels of resistance to RIF; therefore, mutations that are known to result in low-level resistance to RIF are reported with the corresponding comments, warning submitters that such strains may test susceptible by phenotypic assays.

Q: What does it mean when a mutation with "uncertain significance" is detected?

A: A mutation could be categorized as uncertain significance in two cases:

1) The mutation has been previously seen in the WHO catalogue (v.1) with some pre-existing data on correlation with the phenotype available; however, it is insufficient to make a statistically significant prediction of either resistance or susceptibility.
2) The mutation has not been seen previously, but it is covered by an expert rule that allows the interpretation of mutations that are found within genomic regions for which mechanisms of resistance are well understood.
3) The mutation has not been seen previously, but it is a non-synonymous mutation, i.e. leads to an amino acid change; therefore, its effect on drug resistance cannot be ruled out. For any questions regarding the WGS-DST or tNGS-DST results and the interpretation logic applied in each individual case, please reach out to TB DST RC (Matthew.Sylvester@cdph.ca.gov, Varvara.Kozyreva@cdph.ca.gov, CDPHTBDST@cdph.ca.gov).

We recommend that cases of "uncertain" mutations undergo confirmatory phenotypic DST for the corresponding drug, either at TB DST RC or another laboratory. For the drugs for which pDST is not available at TB DST RC, we can assist with referral to other labs.

The determination of treatment regimens and/or discontinuation of treatment should be made based on a combination of available molecular and phenotypic DST data, as well as other laboratory and clinical data. Please contact your state TB program (https://www.cdc.gov/tb/php/tb-programs/index.html) and TB Centers of Excellence (TB COE) (877-390-6682; https://www.cdc.gov/tb-programs/php/about/tb-coe.html) for the consultation if you have any questions about clinical interpretation of your laboratory results and clinical decision-making.

Q: How do I interpret the results if an isolate has discrepant phenotypic and sequencing-based DST results? Specifically, what do I do if phenotypic result for pyrazinamide (PZA) is resistant, but no mutation was detected?

A: Discrepancies between sequencing-based DST (WGS/tNGS) and phenotypic results are possible due to a number of factors, including but not limited to:

- Resistance-conferring mutations in genomic regions not covered by the sequencing-based DST assay.
- Unknown mechanisms of resistance.
- Low level heteroresistance that is below the sequencing assay limit of detection (LOD).
- Propensity of phenotypic DST for some drugs for false-resistance or false-susceptibility.

Specifically, phenotypic testing of PZA using the BACTEC MGIT system is prone to poor reproducibility and false-positive results; this may lead to discrepancies such as when no pncA mutations are detected by sequencing but the strain tests phenotypically resistant. There are a number of high-confidence mutations in the pncA gene that confer resistance to PZA. PZA resistance can be also conferred by mutations in secondary (non-pncA) targets, but their clinical significance is less clearly understood. False resistance is particularly likely for isolates that test phenotypically PZA-monoresistant without pncA mutations. Due to the issues with the phenotypic method for PZA DST and a low pre-test probability, predicted resistance based on pncA sequencing results are more reliable in cases of phenotypic PZA monoresistance. However, it is important to take into account clinical data and other laboratory results. In case of discrepant phenotypic and sequencing-based results for any drugs, please contact TB DST RC (Matthew.Sylvester@cdph.ca.gov, Varvara.Kozyreva@cdph.ca.gov, CDPHTBDST@cdph.ca.gov), your state TB program (https://www.cdc.gov/tb/php/tb-programs/index.html), and TB Centers of Excellence (TB COE) (877-390-6682; https://www.cdc.gov/tb-programs/php/about/tb-coe.html) for consultation and resolution of the discrepancy.

Q: What is the difference between the “No high confidence mutations detected" and “No mutations detected" results that appear on the report?

A: We report mutations for which phenotypic data is available from the World Health Organization or other reputable sources or those that are covered by “expert rules” based on prior knowledge of mechanisms of resistance in TB. Additionally, we report non-synonymous mutations that are NOT found in the current version of WHO catalogue, since their effect on drug resistance cannot be ruled out. To differentiate between genotypes which both are interpreted as “No mutations associated with resistance to XXX detected” we offer the following distinctions:

- “**No mutations detected":** when the gene does not have any mutations (i.e. “wild-type”, WT)
- **“No high confidence mutations detected":** gene contains mutations that are not likely to cause resistance. A “no high confidence mutations detected” genotype could result from a gene containing either:
    - Synonymous mutations;
    - Non-synonymous mutations that are present in the WHO catalogue and are known NOT to be associated with resistance (i.e. neutral mutations);
    - Mutations in promoter regions or non-protein encoding genes that are NOT in the WHO database and are NOT covered by expert rules.

Q: Where can I get information on the number of isolates with a given mutation previously tested Resistant vs. Susceptible?

A: At TB DST RC, we utilize the WHO "Catalogue of mutations in *Mycobacterium tuberculosis* complex and their association with drug resistance" as a basis for our mutation interpretations. It contains information on individual mutations in the TB genome, the number of samples encountered that harbor this mutation, and its association with phenotypic resistance/susceptibility. TB DST RC is currently using the WHO v.1 (2021) database of mutations, but for the reference purposes, we recommend referring to the WHO v.2 (2023) catalogue of mutations. TB DST RC will revalidate our reportable interpretations based on the WHO v.2 catalogue in the near future.

The searchable tables with all described mutations are available as supplementary material (WHO-UCN-TB-2023.7-eng.xlsx [Link; click "View raw" to download]). It is recommended to review Columns "Present_SOLO_R" and "Present_SOLO_S" for the number of strains in the WHO dataset possessing a single given mutation, in the absence of other resistance-conferring mutations, that tested resistant or susceptible to the corresponding drug, respectively. One of the particularly useful values for evaluating the likelihood that a mutation causes resistance is the "PPV|SOLO_lb" value (positive predictive value for resistance); the higher the value, the more likely the presence of this mutation is associated with resistance.

Be aware that in WHO v.2 catalogue of mutations, the nomenclature for *inhA* / *fabG1* mutations has changed and all mutation positions are now displayed in relation to *inhA*. For example, a mutation previously known as *fabG1* c.-15C>T is now listed as *inhA* c.-777C>T in the WHO v.2 catalogue. Information about the former alias for such mutations is available in WHO v.2 tables in the Comments section.

Q: When the WGS/tNGS-DST report says "No sequence" for a given gene, what does it mean? Should I expect results at a later time?

A: If the interpretation for the given drug (e.g. moxifloxacin) indicates "Pending Retest" and one of the associated genes (e.g. *gyrB*) indicates "No sequence", then this is a preliminary report and the sample will be resequenced to obtain the valid *gyrB* sequence. If there is no sequence for the gene and the interpretation for the drug states: "Not all targets could be sequenced; resistance to moxifloxacin cannot be ruled out", this means it is a final report and no additional report will be released. In the latter case, the issues are likely caused by the contamination or suboptimal quality of the sample; submitter will have an option to resubmit.
It is also possible to have a resistance-conferring mutation detected in one of the genes (e.g. *gyrA*) and have unsuccessful sequencing in another gene (*gyrB*) associated with the same drug (moxifloxacin); in that case, the report may be finalized without repeated sequencing, since the sequence of *gyrB* gene in this particular example would not affect the interpretation for moxifloxacin. In this case, no follow-up reports will be issued.

Q: Are synonymous mutations and mutations known not to be associated with resistance reported on the WGS/tNGS-DST report?

A: Generally, synonymous mutations (ones that do not lead to amino acid change) and non-synonymous mutations that are known to be neutral (not associated with resistance) are not displayed on MTBC WGS/tNGS DST report since they do not have an effect on resistance. Exceptions are:

1. Synonymous mutations within the rifampicin resistance determining region (RRDR) of the *rpoB* gene (codons 426-452) are reported because they have a potential to cause false-positive results in real-time PCR assays used for RIF resistance detection such as GeneXpert MTB-RIF.

Either WGS or tNGS can resolve such false-positive results; hence, we recommend submitting isolates determined to be resistant by PCR methods that cannot differentiate synonymous and nonsynonymous mutations for sequencing-based DST for confirmation.

2. The synonymous mutation p.Leu203Leu in *fabG1*, even though silent, confers resistance to isoniazid and is reported.