

# Issues in *Mycobacterium tuberculosis* Complex (MTBC) Drug Susceptibility Testing: Rifampin (RIF)

## BACKGROUND

Rifampin (RIF), the most commonly used member of the rifamycin drug class, is the cornerstone of the first-line regimen used for treatment of drug-susceptible *Mycobacterium tuberculosis* complex (MTBC) due to its potent bactericidal activity. RIF can kill MTBC rapidly by inhibiting the DNA dependent RNA polymerase  $\beta$ -subunit encoded by the *rpoB* gene. The effectiveness of treatment for tuberculosis depends on many factors including the bacterial strain, its metabolic activity, the bacterial burden, pharmacodynamics of the drugs, the site of infection, and the compliance of the patient. Different drugs in a treatment regimen are believed to act on different populations of bacteria in the lesions. RIF is most effective against the dormant bacteria that have short spurts of metabolic activity or active growth because it can kill readily (within 15-20 minutes) unlike other commonly used drugs.<sup>1</sup>

Rifampin stops MTBC by inhibiting the elongation of messenger RNA, ultimately halting transcription. Mutations in the MTBC *rpoB* gene cause changes in the structure of the  $\beta$ -subunit which can lead to various degrees of sensitivity to RIF.<sup>2-4</sup> The majority of RIF-resistant clinical MTBC isolates contain mutations in the *rpoB* gene.

Wild-type strains of MTBC which have not been exposed to RIF are highly susceptible, with minimal inhibitory concentrations (MIC) in the range of 0.125  $\mu\text{g}/\text{mL}$  to 0.5  $\mu\text{g}/\text{mL}$  in the BACTEC™ MGIT™ 960 system (Becton Dickinson). Resistant strains tend to have much higher MICs, most commonly around 8  $\mu\text{g}/\text{mL}$  or as high as 50  $\mu\text{g}/\text{mL}$ .<sup>5,6</sup> Therefore, in the majority of cases, a test concentration of 1  $\mu\text{g}/\text{mL}$  allows discriminations between the majority of susceptible and resistant strains.<sup>7</sup>

In addition to traditional growth-based drug susceptibility testing (DST), molecular detection of DNA mutations can provide valuable information to predict drug resistance, particularly for RIF. Mutations that alter the amino acid sequence—also known as non-synonymous mutations—in the 81-bp “hot spot” known as the RIF resistance determining region (RRDR) of the *rpoB* gene are almost always associated with resistance.<sup>8,9</sup> It has been reported that collectively 96% of RIF-resistant MTBC strains have a mutation within the RRDR of the *rpoB* gene with a limited number of mutations outside the RRDR conferring resistance to RIF.<sup>8,10</sup>

Historically, mutations in the *rpoB* gene of MTBC have been referenced by a numbering system based on the *E. coli* genome. There has been an international movement to change the numbering system to be based on the *M. tuberculosis* (H37Rv) reference genome.<sup>11</sup> We will use MTBC numbering throughout this document (see Figure 1).

However, there are isolates with non-synonymous mutations in the RRDR which are susceptible to RIF by use of growth-based DST methods or have only slightly elevated MIC values close to that of the critical concentration used for testing. Isolates with these results are considered to have low-level resistance to RIF. The clinical impact of these mutations, also referred to as “disputed mutations” is not fully understood, but there is some evidence in the literature that these mutations can be associated with resistance *in vivo*.<sup>12,13</sup> Examples of mutations conferring low-level RIF resistance include: Leu430Pro, Asp435Tyr, His445Asn, His445Leu, His445Cys, Leu452Pro and Ile491Phe (formerly codons 511 516, 526, 533, and 572, respectively, using the *E. coli* numbering system).

As mentioned previously, there are RIF resistant isolates that have mutations outside of the RRDR. Therefore, molecular approaches that do not assess mutations outside of the RRDR, such as codons 170, 250, 299, 482, and 491,<sup>14</sup> may be insufficient to detect all molecular markers of RIF resistance. An additional challenge with molecular detection of resistance is that a strain may be identified as RIF-resistant due to a mutation that does not alter the amino acid (also known as a synonymous mutation or silent mutation) in the RRDR, which does not confer resistance.

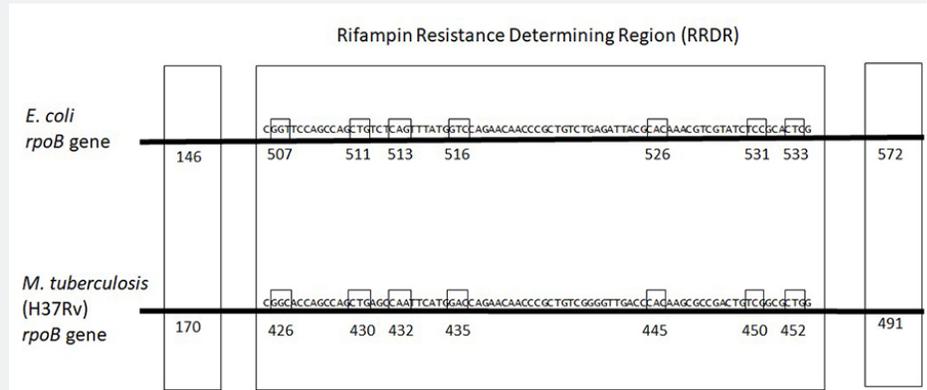
Case studies described in the literature report similar rates of treatment failure in patients receiving RIF therapies that were infected with strains containing certain *rpoB* mutations associated with low-level resistance described above as compared to patients infected with strains containing mutations that confer high-level RIF resistance.<sup>15</sup> Of note, there is considerable variability of phenotypic test results with MTBC strains containing these *rpoB* mutations associated with low-level RIF resistance, as they can often test as susceptible, especially in automated liquid culture systems.<sup>12,13,16</sup> Expert consultation should be sought to consider the treatment regimen for these patients.

**Figure 1. Alignment of 81-bp RRDR of *E. coli* and *M. tuberculosis* complex**

The *rpoB* mutation is now named using the *Mycobacterium tuberculosis* codon system.

**Converting from *E. coli* to MTBC within the RRDR:** Subtract 81 from the old/*E.coli* codon number to obtain the new/MTBC codon number. Example: Codon 526-81= 445 in the new/MTBC numbering scheme.

**Converting from *E. coli* to MTBC outside the RRDR:** Add 24 to the old/*E.coli* codon number to obtain the new/MTBC codon number. Example: Codon 146+24=170



The National TB DST Reference Center has switched to the using the MTBC numbering and the CDC MDDR Service is working to transition to this approach as well.

The seven codons in the RRDR frequently associated with RIF resistance, using the annotation system specific to each species are shown. Additionally, the two most common mutations found outside of the RRDR are also included. Note *M. tuberculosis* numbering is -81 codons from the *E. coli* numbering with the exception of the 146/170 codon. Adapted from Andre, 2017 et al.<sup>11</sup>

## PRACTICAL LABORATORY ISSUES

### Growth-based Phenotypic Rifampin Drug Susceptibility Testing and Test Methods

Current growth-based DST methods for RIF in the US include the agar proportion (AP) method using the Clinical and Laboratory Standards Institute (CLSI) recommended critical concentrations (CLSI M24 and M62) on either 7H10 or 7H11 Middlebrook medium, commercial automated broth systems: BACTEC™ MGIT™ 960 with the BACTEC™ SIRE Drug kit (Becton Dickinson) and VersaTREK™ Automated Microbial Detection System with the VersaTREK™ Myco Susceptibility kit (TREK Diagnostic Systems, Thermo Scientific™); and a microdilution plate method, Sensititre™ *Mycobacterium tuberculosis* MIC Plate (Thermo Scientific™). For ease of use in the document we will use the terms “MGIT assay” to mean the BACTEC™ SIRE Drug kit performed on the BACTEC™ MGIT™ 960 and the term “Myco assay” to refer to the VersaTREK™ Myco Susceptibility kit being performed on the VersaTREK™ Automated Microbial Detection System (Table 1). Of these, only the BACTEC™ MGIT™ and VersaTREK™ systems have FDA-cleared assays to detect RIF resistance. The AP method is considered the reference method and the Sensititre™ assay is for research use only (RUO).

AP is not without its challenges. For example, variability among laboratories may be introduced with the production of drug-containing agar plates and the oleic albumin dextrose catalase (OADC) commercial growth supplement used in the preparation of the agar medium that may vary in purity from lot-to-lot and impact the activity of the drugs. Few clinical laboratories use the AP method because of the complexity of preparing the drug plates and the long turn-around time.

Liquid broth systems are the recommended, and most commonly used, method for first-line DST in clinical laboratories, including RIF, because of the shorter incubation times required for obtaining results as compared to AP. Critical concentrations

for RIF DST are 1.0 µg/mL for Middlebrook 7H10 and 7H11, the MGIT assay and the Myco assay. One comparison between MGIT and Myco assays found 100% correlation between the two assays. However, it was noted that when needles are used to inoculate Myco assay bottles there could be a decrease in the possibility of contamination but increase the risk of occupational transmission.<sup>17</sup> The manufacturer also offers a modified Myco assay bottle with a screw cap which eliminates the need for a needle at this step.

One commercial method for the determination of MICs is the Sensititre™ *Mycobacterium tuberculosis* MIC Plate. This method is a broth microdilution assay, consisting of a 96-well microtiter plate containing twelve antimicrobial agents at appropriate dilutions with RIF being tested at a range of 0.12 µg/mL to 16 µg/mL. There are no CLSI established interpretive break points for the assay and the manufacturer does not provide interpretive criteria for RIF. The manufacturer's protocol states that growth from 7H10 agar should be used for preparing the inoculum, followed by incubation, and checking for growth at 10 days and plates can be read from 7-21 days post-inoculation. Although the turnaround time for this method is longer than the MGIT assay, it has the advantage of reporting susceptibility results for all 12 drugs at one time with most plates being able to be read from 10-14 days.<sup>18-20</sup>

## **Molecular-based Genotypic Rifampin Drug Susceptibility Testing and Test Methods**

Molecular-based assays provide rapid DST results for RIF compared to growth-based methods. Molecular testing methods for detection of mutations in the RRDR associated with resistance to RIF include the Xpert® MTB/RIF (Cepheid, FDA market authorization), the MTBDR<sub>plus</sub> line probe assay (Hain LifeScience, RUO) and other laboratory developed tests (LDT) utilizing real-time PCR, DNA probe or DNA sequencing methods, or a combination of these. These assays can be performed rapidly and directly on the specimen or on isolates. The incorporation of these into testing algorithms and for patient management has been described.<sup>21,22</sup>

Molecular-based assays have some associated challenges and issues. For the majority of *rpoB* gene mutations, a high correlation with growth-based DST is found, but results from molecular assays can be discordant with phenotypic results in some cases. For the probe-based method and Xpert® MTB/RIF, there are known issues with false-positive resistance detection due to identification of synonymous or silent mutations.<sup>23,24</sup> Additionally, the Xpert® MTB/RIF assay has been noted to have a number of limitations. Despite its excellent sensitivity in tests of smear-positive sputum samples, it is somewhat less sensitive when testing smear-negative sputum and in some types of extrapulmonary samples (Xpert® MTB/RIF is not FDA market authorized for these sample types), which are known to contain lower levels of bacilli than pulmonary samples. The assay also has limited capacity to detect RIF resistance associated mutations in mixed samples.<sup>25,26</sup> The Xpert® MTB/RIF assay may also generate occasional false-positive RIF resistance prediction for paucibacillary samples due to delays in the real-time signal generated by assay probes D and E.<sup>27</sup> Xpert® MTB/RIF detection of the *rpoB* silent mutation (Phe431Phe, formerly Phe514FPhe) as conferring RIF resistance has also been reported.<sup>28</sup> For this reason, and in general, it is recommended that DNA sequencing be performed to confirm mutations for this assay and for all other non-sequence based molecular assays. If this cannot be performed at the laboratory performing the assay, samples may be sent to CDC's [Molecular Detection of Drug Resistance \(MDDR\) Program](#).

A new assay, not currently available in the US, has been developed to improve the limitations of the Xpert® MTB/RIF assay, the Xpert® MTB/RIF Ultra assay (Ultra). Initial evaluations indicate improved sensitivity and more definitive identification of RIF susceptibility and resistance.<sup>29</sup> However, similar to the Xpert® MTB/RIF assay, it does not detect potential RIF resistance conferring mutations outside the RRDR.<sup>14</sup>

Similarly, with sequence-based methods used as a stand-alone test or to confirm PCR or probe-based testing, the test or analysis pipeline must differentiate between mutations that are known to confer RIF resistance, those that predict resistance to RIF and those that are not known to predict resistance to RIF.<sup>30-32</sup>

The available testing can be divided into growth-based and molecular-based DST methods (Table 1).

**Table 1. Growth-based and Molecular-based Drug Susceptibility Methods**

Growth-based Method	Determination of Resistance	Regulatory Status (US)
Agar Proportion 7H10	The number of colony forming units (CFU) growing on medium containing RIF at the critical concentration of 1.0 µg/mL compared with the number of CFU growing on drug-free medium	LDT
Agar Proportion 7H11	The number of CFU growing on medium containing RIF at the critical concentration of 1.0 µg/mL compared with the number of CFU growing on drug-free medium	LDT
BACTEC™ MGIT™ 960 SIRE Drug Kit	Growth in the presence of RIF at the critical concentration of 1.0 µg/mL	FDA-cleared
VersaTREK™ Myco Susceptibility	Growth in the presence of RIF at the critical concentration of 1.0 µg/mL	FDA-cleared
Sensititre™ <i>Mycobacterium tuberculosis</i> MIC Plate	The lowest concentration (range 0.12-16 µg/mL) that shows no visible growth	RUO*
Molecular-based Method	Determination of Resistance	Regulatory Status (US)
Xpert® MTB/RIF	Detection of mutations in the RRDR through probe binding	FDA-market authorized
MTBDR <i>plus</i> line probe assay	Detection of mutations in the RRDR associated with RIF resistance by lack of hybridization to wild-type sequence probes and/or hybridization to probes containing known mutations visualized on a test strip.	RUO*
Sanger sequencing	Detection of mutations in <i>rpoB</i>	LDT
Pyrosequencing	Detection of mutations in <i>rpoB</i> and in the RRDR	LDT
Targeted Next-generation Sequencing (NGS)	Detection of mutations in <i>rpoB</i> e and its promoter region	LDT
Whole-genome sequencing (WGS)	Detection of mutations in <i>rpoB</i> while assessing the entire genome for other genetic predictors of drug-resistance	LDT
*Assays are commercially available as RUO but can be validated for clinical testing		

## Considerations for Drug Susceptibility Testing for Rifampin

Growth-based DST with solid and liquid methods has been well studied for RIF, and a consensus based on a robust assessment of published studies, laboratory experience and expert opinions regarding methodology, critical concentrations, and expected performance is established.<sup>7</sup> With all growth-based methods, particularly the automated systems, it is good practice to ensure inocula do not contain clumps of MTBC and to allow sufficient time for settling of clumps in the broth suspension before inoculation of the drug tubes as some reports have shown improvement in reproducibility of results.<sup>33</sup> One complication to DST interpretation for RIF is the potential for discrepant results among the increasing number of different DST methods available, including the rapid molecular methods. Additionally, in some settings, detection of RIF resistance serves as a reliable (although not complete) proxy of multi-drug resistant tuberculosis (MDR-TB). The advantages of rapidly identifying RIF resistance, particularly through molecular methods, includes earlier identification of patients on inappropriate first-line regimens, rapid screening of patients at risk of MDR-TB, and early interruption of MDR-TB transmission.<sup>7</sup>

A comprehensive review of studies that compared the MGIT assay with other growth-based methods for detecting resistance to RIF showed excellent (98%) sensitivity for the MGIT assay.<sup>7</sup> However, another study found that the MGIT assay was less sensitive than the Lowenstein Jensen proportion method in detecting RIF resistance.<sup>16</sup>

As previously described, one of the major challenges with RIF resistance testing is understanding the clinical impact of *rpoB* mutations associated with low-level RIF resistance. It is recognized that the frequency of such mutations varies by study.<sup>12,30,34–36</sup>

Currently, efforts are ongoing to better characterize the frequency and impact of these mutations in the US. It is important to note that these mutations can easily remain undetected using the current standard DST methods, including the rapid, broth-based systems. Therefore, if only growth-based DST is used, some MTBC isolates that contain these mutations will be classified as RIF susceptible. Case reports in the literature suggest that treatment failure from RIF has been reported with isolates containing these types of mutations.<sup>12,15</sup> This has recently become an area of significant interest, and additional information about these mutations is rapidly becoming available. As more is learned, it could impact or alter the current DST methods and/or lead to the development of new approaches to more accurately identify RIF resistance.

Another diagnostic challenge and consideration is the identification of synonymous/silent mutations which do not alter the *rpoB* amino acid sequence and are not associated with RIF resistance. However, certain assays such as Xpert® MTB/RIF detect these as mutations and are reported as RIF-resistant. Mutations detected by probe B of the Xpert® MTB/RIF are more likely to be associated with a silent/synonymous mutation than those mutations detected by other probes. Other methods such as DNA sequencing are capable of identifying these as silent mutations and negate the possibility of reporting of these mutations as conferring RIF resistance. For this reason DNA sequencing is recommended to confirm probe based detection of RIF-resistant mutations.

To assure detection of RIF resistance, some laboratories have implemented testing RIF by broth microdilution (MIC), sequencing methods<sup>30,37,38</sup> or longer MGIT assay incubation times<sup>39</sup> which all enable detection of the presence of RIF resistance when low-level resistance caused by certain *rpoB* mutations are present.

## Quality Assurance

CLIA-certified laboratories must participate in a CLIA-approved proficiency testing (PT) program to satisfy regulatory requirements for DST performance. If a qualified program is not available a suitable alternative must be implemented such as inter-laboratory comparison. In the US, the College of American Pathologists provides PT for RIF and other antituberculosis drugs; however, their program provides just two challenge isolates per year and only includes pan-susceptible strains. Other commercial PT programs, such as American Proficiency Institute, do provide PT challenges for MTBC DST. The US Centers for Disease Control and Prevention (CDC) offers the [Model Performance Evaluation Program](#) (MPEP) for MTBC DST, which is not a formal, graded PT program but could be used as an adjunct to the laboratory's regulatory PT program. The MPEP Program is an educational self-assessment tool offering five MTBC isolates per challenge of which both drug resistant and drug susceptible isolates are included. It provides an opportunity to compare results to those obtained by other participants using the same methods.

## IMPACT ON CLINICAL OUTCOMES

In 2017, 97 new MDR-TB cases were reported in the US, 1.6% incidence of all existing cases.<sup>40</sup> RIF resistance is often found in combination with resistance to isoniazid (INH) and resistance to at least these two drugs is the definition of MDR-TB. For this reason, mutations in the RNA polymerase B subunit or *rpoB* gene (which confers RIF resistance) are often used as a surrogate for detecting MDR-TB. Monoresistance to RIF has also been reported in the US at rates of <1%<sup>41</sup> and in other countries at rates between 7- 33%,<sup>42,43</sup> with recent reports suggesting that detection of RIF should no longer be used to predict MDR-TB in some countries.<sup>44</sup>

The clinical impact of *rpoB* gene mutations associated with low-level RIF resistance on TB patients being treated in North America is limited.<sup>15</sup> Clinicians can be made aware of the presence of a low-level *rpoB* gene mutation if detection of the mutation by Xpert® MTB/RIF is followed by *rpoB* gene sequencing as recommended elsewhere in this document. Because RIF retains some *in vitro* activity in the presence of these mutations, clinicians have included a rifamycin in the treatment regimen, while not relying on RIF as they normally would and using an increased dosage.<sup>45-47</sup>

## Rifabutin

Rifabutin (RFB) is a member of the rifamycin class of drugs and is recommended for TB treatment in RIF-susceptible HIV-co-infected patients under anti-retroviral therapy. Certain *rpoB* gene mutations are associated with a phenotype of RIF resistant, RFB susceptible, especially mutations at codons 435 and 445 (formerly codons 516 and 526).<sup>48</sup> Some suggest that RFB DST should be performed when RIF resistance is observed.<sup>49</sup> Test concentrations for RFB should be 0.5 µg/mL in the MGIT assay or AP.<sup>48</sup> The clinical utility of RFB when such a mutation is found has not been established, however, RFB has been used in these situations based on its *in vitro* activity.<sup>49,50</sup>

It should be noted that certain mutations in the RRDR of the *rpoB* gene appear to confer cross-resistance to both RIF and

RFB.<sup>10,51-54</sup> The mutations Ser450Leu, His445Tyr and His445Asp (formerly Ser531Leu, His526Tyr, and His526Asp) are most common and are found in isolates from a majority of MDR-TB patients. Berrada et al. demonstrated the association of various RRDR mutations with the differential expression of phenotypic resistance or susceptibility to RIF and RFB as measured by the MGIT assay.<sup>55</sup> These data may serve as a starting point to establish a database containing MICs of RFB and RIF associated with specific *rpoB* mutations, which will evolve as new mutations are detected and new MICs added.<sup>55</sup> Clinical studies to assess RFB treatment in certain MDR-TB cases are needed.<sup>55</sup> MDR-TB is often difficult to treat, therefore, development of a collection of evidence regarding the potential use of RFB for treatment of RIF-resistant tuberculosis is desirable.

## AREAS OF ONGOING RESEARCH

There is a low prevalence of RIF resistance in the US and limited data available on the clinical implications of certain *rpoB* mutations means that there is disagreement about how to interpret these mutations and treat patients. It may be important to assess other published studies. Clinical impact of *rpoB* mutations in patients from Bangladesh or Africa could conceivably be different from impact on patients in North America, due to differences in nutritional status, disease severity at initiation of treatment, variations in drug quality, and/or differences in the quality of supervision of the directly observed therapy. Studies in North America may take longer to perform, because of a lower prevalence of RIF resistance. However, approximately 10% of *rpoB* mutations found in the US are associated with low-level resistance (personal communication, James Posey). Continued assessment of the frequency of occurrence of *rpoB* mutations associated with low-level RIF resistance in North America should be performed, in order to assess the cost-effectiveness of potential changes in the DST protocols for rifamycins.

A recent analysis concluded that molecular-based genotypic DST should be used to replace phenotypic results (MGIT assay) when low-level mutations are identified due to a delayed growth in the MGIT assay.<sup>39</sup> However, additional research has shown that the MTBDR*plus* line probe assay could be used as a complementary test to confirm RIF DST results obtained using culture-based testing to rapidly assess the type RIF mutations present.<sup>56</sup>

Novel molecular-based approaches to detect mutations associated with RIF resistance are being developed by manufacturers and available outside of the US. The BD MAX™ MDR-TB Panel is a molecular assay for diagnosis of TB and detection of mutations associated with RIF and INH resistance. While the assay has received a CE mark there is very limited data about the assay performance at the time this document was published. Initial evaluations of the Xpert® MTB/RIF Ultra assay (Ultra)—endorsed by the WHO but not currently available in the US—indicate improved sensitivity and more definitive identification of RIF susceptibility and resistance. Evaluations performed to date describe the changes in the Ultra results that may resolve discordance between a RIF-resistant Ultra result and a low-level RIF susceptible phenotypic result due to a *rpoB* mutation conferring low-level RIF resistance.<sup>14,29</sup> Ultra has the capacity to utilize  $\Delta T_m$  values in the exported results that can discriminate between “disputed mutations” and known mutations at codons 428, 430, 431, 432, 434, 435, 441, 445, 446, and 452.<sup>14</sup>

Another important area of ongoing research is the development of whole-genome sequencing (WGS)<sup>30,37,57,58</sup> and targeted next generation sequencing (NGS)<sup>59-61</sup> approaches that provide comprehensive analysis of the MTBC genome to more accurately assess genotypic DST. It has been suggested that sequencing of all mutations with a method such as WGS offers advantages over line-probe assays and other commercial molecular assays.<sup>30,37,62,63</sup> There is value in detecting all mutations as this can result in a higher sensitivity and provide more definitive data on which TB drugs to utilize, but these methods typically require cultured material and can take many days to test and report findings compared to currently available PCR and pyrosequencing methods. WGS genotypic DST can be accomplished for additional and even novel drugs at no additional cost, contingent only on the knowledge base of characterized mutations and this testing data could be helpful when designing new treatment regimens.<sup>37</sup> Reports of shorter turnaround-times compared to growth-based DST with results available 7-8 days from a positive culture have also been described with the implementation of this testing.<sup>30,62,64</sup> Comprehensive WGS approaches and evaluation of data on low-level resistance mutations may continue to inform the best practices for treatment when strains harboring these mutations are present. Targeted NGS enables the detection of a significantly large enough portion of relevant mutations and could serve a similar role as WGS but the added benefit that may be applied directly to specimens, provide results more quickly than WGS, and at a lower cost.<sup>59-61</sup>

Studies describing the potential to perform next generation sequencing (NGS) directly from a specimen have been published<sup>59,60,65</sup> including a commercially available product, the Ion AmpliSeq™ TB panel (Life Technologies, Carlsbad, CA, US), that provides full-length gene analysis using ion semiconductor NGS.<sup>65,66</sup> Another RUO system, Deeplex® Myc-TB (Genoscreen, Lille, France), uses deep sequencing of a 24-plexed amplicon mix for simultaneous identification of mycobacterial species, genotyping, and prediction of drug resistance of MTBC. This includes 18 gene regions associated with resistance to 15 first and second-line drugs.<sup>67-70</sup> The ability to provide comprehensive detection of mutations and derive additional information on the

MTBC genome before culture is available could have a dramatic impact on TB diagnosis and patient management. Approaches for the direct sequencing of sputum are currently slower than rapid methods for detecting resistance, but can assess susceptibility to a wider range of drugs and concurrently provide relatedness among MTBC strains, supporting infection control efforts to understand transmission.

Finally, studies and new data continue to emerge about possibly increasing the RIF standard dose. If a change in treatment recommendation were to occur, this could impact protocols for testing and reporting RIF by molecular and phenotypic methods.<sup>13,45,71</sup> Additionally, if these recommended RIF doses are increased, the significance of low-level resistance could be impacted as well.

## GUIDANCE

Laboratories should consider the following:

- Use of rapid detection of MTBC and RIF resistance directly on the clinical specimen, or alternative molecular method for screening for MDR-TB, should be encouraged in all cases of TB-suspect patients. A MDR molecular screening test should indicate when there is a mutation in the RRDR. DNA sequencing should be performed promptly to confirm resistance and identify if the mutation is a silent or low-level resistance mutation.
- Growth-based DST may be interpreted as susceptible for some strains of TB that have low-level resistance mutations detected by a molecular method. In these cases laboratories should be available for consultation on test interpretation.
- Care must be taken to follow instructions and assure adequate vortexing of inocula to break up clumps of MTBC and to allow sufficient time for settling of clumps in the suspension before inoculation of the drug tubes to avoid potential issues with false positive or negative results.
- If your laboratory is unable to perform molecular testing for RIF, samples can be referred to a jurisdictional public health laboratory for testing and/or those laboratories can refer to the following services:
  - CDC [Molecular Detection of Drug Resistance Service](#) (MDDR) which provides rapid algorithm-based testing and other comprehensive testing services at no cost to all public health laboratories.
  - [The National Public Health Laboratory Drug Susceptibility Testing \(DST\) Reference Center for \*Mycobacterium tuberculosis\*](#) provides rapid algorithm-based testing at no cost to enrolled public health laboratories. Enrollment is restricted to public health laboratories performing TB DST on fewer than 50 isolates/year.
- When a low-level *rpoB* mutation is found, the clinician(s) caring for the patient should be encouraged to seek expert consultation. Persons infected with strains with these mutations may be associated with diminished treatment success.

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