

Laboratory Considerations for Use of Cepheid Xpert® MTB/RIF Assay

This document is intended to guide laboratories on integrating the Cepheid Xpert® MTB/RIF assay into existing TB testing practices.

The introduction of nucleic acid amplification (NAA) testing into TB testing algorithms has brought the promise of accurate and time efficient methods for detecting *Mycobacterium tuberculosis* bacteria and mutations associated with drug resistance. With the availability of rapid NAA testing, such as the Xpert® MTB/RIF assay, test results can be reported weeks earlier than culture, leading to improved patient management and outcomes, and preventing unnecessary use of resources. However, it is **strongly recommended** that NAA tests be performed and interpreted in the context of a comprehensive testing algorithm that includes AFB smear and culture as well as drug susceptibility testing (DST) to maximize its benefit to patient management.

The Centers for Disease Control and Prevention (CDC) recommends that NAA testing be performed on at least one respiratory specimen from patients who have a moderate or high suspicion of having pulmonary TB¹. The FDA market authorization of the Xpert® MTB/RIF assay provides the opportunity for more laboratories to offer rapid diagnosis of pulmonary tuberculosis.

In July 2013, the FDA granted Market Authorization to the Cepheid Xpert® MTB/RIF assay. This NAA test can simultaneously identify *Mycobacterium tuberculosis* complex (MTBC) and genetic mutations associated with resistance to rifampin from raw sputum and concentrated sputum sediments. The assay utilizes a self-contained, disposable cartridge that can be used on Cepheid's fully-automated GeneXpert® Instrument Systems. The convenience and automation of this system has the potential to provide rapid access to patient results.

Key points laboratories and clinicians must consider when adopting or performing the Xpert® MTB/RIF assay:

- Specimens received in the laboratory for TB testing are strongly recommended to receive both AFB smear and culture regardless of the NAA test result to confirm the presence or absence of MTBC.
- Specimens in which rifampin resistance is detected are strongly recommended for immediate referral to your state or local public health laboratory to receive confirmation of rifampin resistance by DNA sequencing.
- Isolates are required for all positive MTB patients in order to perform culture-based susceptibility testing and for genotyping.
- If AFB smear and/or culture are not available in-house, specimens should be sent to a reference laboratory.
- For optimal interpretation of results, perform AFB smear and culture on the same specimen. If this is not possible, collection of an additional specimen will be required for AFB smear and culture.

Importance of AFB Smear

- AFB smears should be performed on all specimens tested by NAA in order to best interpret the NAA test results as per CDC guidelines¹.
- Due to overnight accumulation of secretions, first morning specimens are more likely to yield better recovery of AFB². If possible, both AFB smear and the Xpert® MTB/RIF assay should be performed on first morning specimens. When first morning specimens are not practical, supervised collection of sputum is recommended.
- According to the package insert, the Xpert® MTB/RIF assay is:
 - 99.7% sensitive and 98.5% specific when compared to culture for smear positive sputum.
 - 76.1% sensitive and 98.8% specific when compared to culture for smear negative sputum³.

Therefore, knowing the AFB smear result in conjunction with a NAA test can better inform clinical decisions. For example, a negative NAA test on a smear positive specimen used in conjunction with patient history and clinical presentation, could contribute to ruling out active TB.

- Due to the decreased sensitivity of the NAA test on smear negative specimens, there is a higher likelihood of false negative results and a negative NAA cannot be used to rule out active TB.
- If there is a true clinical suspicion of TB, the NAA test should be performed regardless of smear status, as recommended by CDC.
 - Patients with HIV and pulmonary TB co-infection have a higher likelihood of being smear negative. Therefore NAA testing is particularly useful in this population. Laboratories should proceed with caution in using the Xpert® MTB/RIF assay for patients with known HIV infection, as performance characteristics in the US population are not currently known.
 - WHO recommendations for use of the Xpert® MTB/RIF assay in populations where HIV and pulmonary TB co-infection may be suspected, are available at:
http://who.int/tb/features_archive/factsheet_xpert.pdf

Importance of AFB Culture

- A negative NAA test result does not exclude the possibility of a positive culture.
- A positive NAA test result does not differentiate between the species of MTBC or determine the presence of other Mycobacteria species.
- Isolates are required for all positive MTB patients in order to perform culture-based drug susceptibility testing and genotyping.
- Xpert® MTB/RIF and AFB smear results should be reported as soon as available.

Importance of Drug Susceptibility Testing

- All MTB positive specimens determined to be rifampin resistant by the Xpert® MTB/RIF assay are **strongly recommended** to have the mutation associated with rifampin resistance confirmed by DNA Sequencing at a reference laboratory or at CDC.

- All MTB isolates, including those that are RIF sensitive, should receive additional DST by culture-based methods to determine the susceptibility patterns of other drugs used to treat TB.
- When suspicion of drug resistance is high, it is recommended to perform molecular methods to detect resistance to other drugs used to treat TB. These methods are available at some reference laboratories.
- The Xpert® MTB/RIF assay detects mutations associated with only rifampin resistance. All MTB positive specimens determined to be rifampin resistant by the Xpert® MTB/RIF assay should be followed up with concurrent first and second line DST performed on additional specimens.
- CDC offers a molecular detection of drug resistance (MDDR) testing service free of charge. They accept NAAT positive concentrated sputum specimens as well as MTB isolates. The service can be accessed by submitting specimens or isolates to your State or Local Public Health Laboratory. Some state laboratories may also perform DNA sequencing to confirm a result of rifampin resistance by Xpert® MTB/RIF assay. For information on submitting specimens to CDC's MDDR service, see the website:

<http://www.cdc.gov/tb/topic/laboratory/UserGuide/submitters.htm>

OR

<http://www.cdc.gov/tb/topic/laboratory/MDDRUsersGuide.pdf>

Summary of Considerations Based on In House TB Testing Capability

If your laboratory performs Xpert® MTB/RIF only...

It is best practice to interpret the results of TB NAA tests in conjunction with an immediate AFB smear result. However, if your laboratory chooses to perform only the Xpert® MTB/RIF assay the following actions must be taken:

- √ **It is strongly recommended that specimen be sent to a reference laboratory for AFB smear and culture as soon as possible regardless of the NAA result.**
 - If there is a sufficient volume of raw sputum, split the specimen and send to a reference laboratory for both concentrated AFB smear and culture. The sample must be split prior to the laboratory mixing a sputum sample with the Sample Reagent (or SR). A sample treated with SR, while very easy to split due to liquefaction, will not be viable for culture.
 - If volume is insufficient, request an additional sputum specimen for AFB smear and culture.
- √ Report results from the Xpert® MTB/RIF assay as soon as available while awaiting culture confirmation.
- √ If RIF resistance is detected, a specimen should be sent to a reference laboratory to confirm the resistance by DNA sequencing as soon as possible.

If your laboratory performs AFB Smear and Xpert® MTB/RIF...

Performing either direct or concentrated smear in conjunction with the TB NAA assay improves your ability to interpret the results. However, laboratories should consider the following:

- √ Direct smear is less sensitive than concentrated smear but may be appropriate when used in conjunction with the Xpert® MTB/RIF assay for laboratories that do not have the capacity to perform concentrated smears.
- √ AFB Smear should only be performed by laboratories that are proficient in the technique and have the appropriate biosafety measures in place.
- √ **It is strongly recommended that specimens be sent to a reference laboratory for AFB culture as soon as possible regardless of the AFB smear and NAA result.**
 - If there is sufficient volume of raw sputum, split the specimen and send to a reference laboratory for culture confirmation. The sample must be split prior to the laboratory mixing a sputum sample with the Sample Reagent (or SR). A sample treated with SR, while very easy to split due to liquefaction, will not be viable for culture.
 - If volume is insufficient, request an additional sputum specimen for AFB smear and culture.
- √ If RIF resistance is detected, a specimen should be sent to a reference laboratory for DNA sequencing to confirm the resistance as soon as possible.
- √ Report results from AFB smear and the Xpert® MTB/RIF assay as soon as they are available while awaiting culture confirmation.

If your laboratory performs AFB Smear, Xpert® MTB/RIF and AFB Culture...

It is best practice to interpret the results of TB NAA tests in conjunction with an immediate AFB smear result. However, if your laboratory chooses to perform only the Xpert® MTB/RIF assay the following actions must be taken:

- √ Report results from the AFB smear and Xpert® MTB/RIF assay as soon as they are available while awaiting culture confirmation.
- √ If RIF resistance is detected, a specimen should be sent to a reference laboratory for DNA sequencing to confirm the resistance as soon as possible.
- √ AFB cultures positive for MTB must be sent to a reference laboratory for culture-based DST and genotyping.

Rifampin Results Reporting Language Guidance

The following guidance is suggested for laboratories reporting RIF resistance detection results from the Xpert® MTB/RIF assay to clinicians. For questions concerning the clinical application of molecular drug susceptibility testing results, clinicians are encouraged to consult their Regional Training and Medical Consultation Center (RTMCC) or clinical TB expert. For information on the 5 regional RTMCCs, see the website: <http://www.cdc.gov/tb/education/rtmc>

Xpert® MTB/RIF Readout	Interpretation	Report* (Suggested Minimal Language)
MTB DETECTED; RIF Resistance DETECTED	MTB target is detected within sample. A mutation in the <i>rpoB</i> gene has been detected. A full first and second line drug panel should be conducted.	MTBC detected. rpoB mutation detected; likely rifampin resistance; Confirmatory testing in progress OR isolate has been forwarded to a reference laboratory for confirmatory testing.
MTB DETECTED; RIF Resistance NOT DETECTED	MTB target is detected within sample. A mutation in the <i>rpoB</i> gene has not been detected.	MTBC detected. No <i>rpoB</i> mutation detected; likely rifampin susceptible.
MTB DETECTED; RIF Resistance INDETERMINATE	MTB target is detected within sample. A mutation in the <i>rpoB</i> gene could not be determined due to insufficient signal detection.	MTBC detected. Insufficient MTB in the sample to allow determination of <i>rpoB</i> mutation result.
MTB Not Detected	MTB target is not detected within the sample.	MTBC not detected.

***RIF Results should be reported prior to culture confirmation.**

Quality Assurance

- The Xpert® MTB/RIF cartridges include internal controls. External controls should be used in accordance with local, state and federal accrediting organizations' requirements as applicable.
- The only CMS approved proficiency testing (PT) program available exclusively for MTB PCR is available through the Wisconsin State Laboratory of Hygiene. The Molecular Microbiology Nucleic Acid Amplification survey offered by the College of American Pathologists includes one MTB PT specimen.
- Currently, there are no CMS approved PT programs for the RIF portion of the Xpert® MTB/RIF assay. According to Clinical Laboratory Improvement Amendments (CLIA), if a laboratory performs a

test for which no CMS approved PT program is available, it must demonstrate the accuracy of this test at least twice a year. Laboratories do this in a variety of ways including sharing specimens or the in-house development of blinded panels from rifampin resistant isolates that the laboratory has retained⁴. The Clinical Laboratory Standard Institute's (CLSI) GP-29A provides other suggestions for demonstrating the quality of laboratory tests when PT programs are not available.

Other Considerations

- The Xpert® MTB/RIF assay is only authorized for use on sputum (either induced or expectorated) specimens. Any other specimen types must be validated by the laboratory as an off label use of the assay.
- The Xpert® MTB/RIF assay has only been authorized for use on sputum samples from untreated patients and patients currently being treated for TB for less than three days, and should not be used as a test of cure.
- The Xpert® MTB/RIF assay has not been FDA authorized for use in specimens from pediatric patients.
- A negative MTB result on the Xpert® MTB/RIF assay does not rule out pulmonary TB. A positive NAA test does not necessarily indicate the presence of viable organisms.

Public Health Impact

In order to control the transmission of TB, it is critical that public health authorities receive rapid notification of new TB cases and suspected drug resistance. When MTB and/or RIF resistance is detected, laboratories should follow state regulations and guidelines on reporting positive results and submitting specimens or isolates to the Public Health Department to ensure initiation of contact investigations and other TB control measures.

Additional Resources

Biosafety in Microbiological and Biomedical Laboratories (BMBL) 5th Edition. HHS Publication No. (CDC) 21-1112

Revised December 2009. <http://www.cdc.gov/biosafety/publications/bmb15/BMBL.pdf>

StopTB.org. Published evidence and commentary on the Xpert MTB/RIF assay. Updated 31 July 2013. <http://www.stoptb.org/wg/gli/assets/documents/map/XpertPublications.pdf>

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