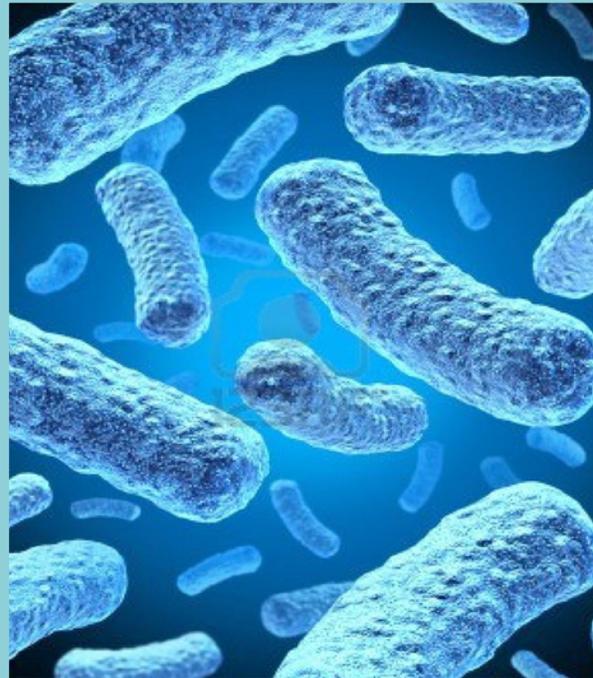
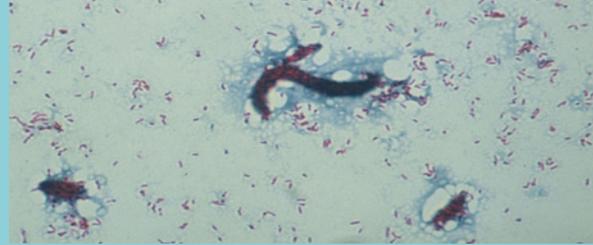


**Suggested Standard
Reporting Language
for Molecular
Detection of
*Mycobacterium
tuberculosis*
Complex (MTBC)
and Mutations
Associated With
Drug Resistance**



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Introduction

While a combination of clinical indicators and conventional testing, including microscopy and growth-based methods, have traditionally been used to diagnose active tuberculosis (TB) caused by *Mycobacterium tuberculosis* complex (MTBC), molecular testing is becoming more frequently utilized. The language used for reporting results from conventional and molecular methods can vary between laboratories with respect to technical terminology and interpretative comments. This can cause confusion or clinical misinterpretation and negatively impact or delay proper patient management decisions. The suggested standard reporting language presented herein reflects the consensus of subject matter experts from the Association of Public Health Laboratories (APHL) Tuberculosis Subcommittee. This reporting language serves as an interim resource as efforts are made to globally harmonize MTBC reporting, specifically as it relates to reporting molecular testing results. The reporting guidance in this document is only suggestive and may need to be adapted or modified depending on factors such as jurisdictional requirements or advances in technology. It is important to remember that molecular testing supplements growth-based methods, and does not replace it. Culture must be performed in addition to molecular testing to confirm cases and provide an isolate for drug susceptibility testing and genotyping.

The suggested standard reporting language emphasizes clear and concise interpretation of results, regardless of molecular assay or target, and applies to all nucleic acid amplification tests (NAAT) for the direct detection of MTBC in clinical specimens, including laboratory developed tests (LDTs) and FDA-cleared, approved or market authorized tests. This document intends to clarify and guide how to report test results to clinicians or submitting agencies as well as recommendations for follow-up testing if appropriate. If a product package insert states or instructs the laboratory how to report the results of the test, the laboratory must use that language as per CLIA requirements. However, the laboratory may add additional interpretive comments to provide clarifying information or suggested next steps for clinicians.

Part I: Molecular Detection of MTBC

There are several molecular detection methods used in the United States including: Amplified *Mycobacterium Tuberculosis* Direct Test (Hologic), Cepheid GeneXpert (Xpert) MTB/RIF, Line Probe Assays (LPA), Real-time PCR and DNA Sequencing. Each of these methods is used for the detection of MTBC from patient specimens and the suggested reporting language encompasses all of these methods. We have provided suggested reporting language (Table 1) for a positive, negative, indeterminate or invalid result. For each of those results we have also provided interpretative comments taking into account the accompanying AFB smear results. The term “indeterminate” is used in this document to indicate that the presence or absence of MTBC cannot be determined. According to CLSI MM03-Ed3,¹ indeterminate is synonymous with equivocal. Additionally, some tests may also have an “invalid” result which would also need to be reported per the package insert.

The terms that are used in your report should be defined within the report to ensure clear communication of the results.

Interpretive comments will vary among laboratories. Comments are intended to provide supporting information, including limitations and recommendations, to aid in clinical decision making and follow-up testing.

The National TB Controllers Association (NTCA) and APHL published a consensus statement on the use of the Cepheid Xpert MTB/RIF[®] assay in making decisions to discontinue airborne infection isolation in healthcare settings which contains additional information regarding the interpretation of Xpert results in the context of AFB Smear.

Available at: http://bit.ly/APHLNTCA_consensus

Regardless of the result, the following additional comment should also be included: “The test results must be interpreted in conjunction with the AFB smear results and other laboratory findings, clinical presentation, radiologic findings, and risk factors for active tuberculosis (TB). Culture results are pending.”

Table 1: Suggested Reporting Language for the Molecular Detection of MTBC^a

Result	Reporting Language	Interpretation in Conjunction with the AFB Smear Results		
		AFB Smear Positive Result	AFB Smear Negative Result	Repeat Testing
Positive	MTBC detected	The patient can be presumed to have active tuberculosis (TB), pending culture results, without additional [name of test] testing. The [name of test] does not determine the viability of organisms and cannot be used to monitor the response to therapy.	Consider repeat [name of test] with an additional specimen to confirm the result of [name of test]. The [name of test] does not determine the viability of organisms and cannot be used to monitor the response to therapy.	N/A
Negative	MTBC not detected	A negative [name of test] does not rule out active tuberculosis (TB). If active TB is clinically suspected consider testing a second specimen (not to exceed a total of two) to confirm the negative [name of test] result.	Currently available NAATs are not sufficiently sensitive to exclude diagnosis of tuberculosis in AFB smear negative patients in whom active TB is suspected. Testing of additional smear negative specimens is not recommended if clinical suspicion is low.	A negative [name of test] does not rule out active TB. With two negative [name of test] the interpretation should be made in conjunction with the AFB smear results. If AFB smear positive and NAAT negative on two tests, infection with nontuberculous mycobacteria (NTM) is likely, pending culture results. If AFB smear negative on two tests, and active TB cannot be ruled out, continue diagnostic evaluation. If two AFB smear results are discordant and NAAT is negative for both, active TB is not likely and infection with NTM is possible, pending culture results.
Indeterminate	Presence or absence of MTBC cannot be determined	An indeterminate [name of test] does not rule out active tuberculosis (TB). Results are inconclusive for MTBC. Additional testing is necessary for clarification of results. Other additional comments: Inhibitors detected, ^b High Ct values may be reflective of assay interference and/or low bacterial burden or insufficient bacterial DNA. ^c		
Invalid ^d	Presence or absence of MTBC cannot be determined	An invalid [name of test] does not rule out active tuberculosis (TB). The sample processing control (SPC) does not meet the acceptance criteria, the sample was not properly processed or PCR was inhibited. Repeat the test following the Retest procedure in the package insert.		

a. Recipients of these results are encouraged to seek expert consultation from the [State TB Control Program](#) and/or the [Regional Training Medical Consultation Center \(RTMCC\)](#) for their use for patient management.

b. Comment should only be used when the molecular detection assay includes an inhibition control.

c. Comment should only be used when a real-time PCR assay that includes a result with cycle threshold (Ct) values.

d. Comments are only applicable for the Cepheid Xpert MTB/RIF assay for detection of MTBC.

Example Test Description Language

Below we have provided a template for a test description for both a laboratory developed test (LDT) and a FDA-approved test as well as a specific example for each. Any modification to an FDA-approved or FDA-cleared test would be interpreted as off-label usage and would also qualify it as a LDT. All of the example reports presume that an AFB smear result is available and is included on the laboratory report with the NAAT result. While not included in the examples below, laboratories may also consider including limitations of the assay. Lastly, validation and verification data should be readily available including the number of specimens and the performance characteristics of the test. These data may be communicated to submitters when the test is implemented or in the description of the test.

Description for a Laboratory Developed Test (LDT)

Template

This test is a [name of test, description of test] for the detection of *M. tuberculosis* complex [DNA, RNA, or nucleic acid] directly from clinical specimens. It is a laboratory developed test and its performance characteristics have been verified by the [name of laboratory]. It has not been approved or cleared by the FDA. The sensitivity of the assay is XX% for AFB smear positive specimens and XX% for AFB smear negative specimens, with specificities of XX% and XX%, respectively.

Specific Example

This test is a real-time PCR assay that detects the insertional element IS6110 that is found exclusively within the *Mycobacterium tuberculosis* complex (MTBC) for the detection of MTBC DNA directly from clinical specimens. It is a laboratory developed test and its performance characteristics have been verified by State Public Health Laboratory. It has not been approved or cleared by the FDA. The sensitivity of the assay is 92% for AFB smear positive specimens and 72% for AFB smear negative specimens, with specificities of 98% and 98%, respectively.

Description for an FDA-Approved Test

Template

This test is a [name of test, description of test] for the detection of *M. tuberculosis* complex [DNA, RNA, or nucleic acid] directly from clinical specimens. It has been approved/cleared/market authorized by the FDA for [specimen types]. Based on [package insert, published data including citations, or testing in our laboratory] the sensitivity of the assay is XX% for AFB smear positive specimens and XX% for AFB smear negative specimens, with specificities of XX% and XX%, respectively.

Specific Example

This test is an Xpert MTB/RIF, which is a qualitative, nested real-time PCR for the detection of *M. tuberculosis* complex DNA directly from clinical specimens. It has FDA market authorization for raw sputum or concentrated sputum sediment from induced or expectorated sputum. Based on the package insert the sensitivity of the assay is 99% for AFB smear positive specimens and 79% for AFB smear negative specimens, with specificities of 97% and 97%, respectively.

Part II: Molecular Detection of Drug Resistance by DNA Sequencing

The suggested reporting language for molecular detection of drug resistance to Rifampin (RIF) and Isoniazid (INH) is intended for laboratories performing sequencing methods such as pyrosequencing, Sanger sequencing or next generation sequencing (NGS) methods for INH or RIF (Table 2). Each of these methods is used for the detection of mutations associated with drug resistance for MTBC and the suggested reporting language encompasses all of those methods. For labs that are performing testing for only RIF using the Xpert MTB/RIF assay please refer to the package insert² for reporting language and the [Interpretation and proposed minimum laboratory report language for results from the Cepheid Xpert MTB/RIF assay – United](#)

[States 2013](#).³ Additionally it should be noted that a positive RIF result from this assay indicating a mutation in *rpoB* should be confirmed by a DNA sequencing method. The table below outlines the elements that should be included in a report for molecular detection of drug resistance. Although not included in the table below, the report should also include the name of the method used. Additional comments can also be included to provide context or further information to the provider or other persons receiving the report. The elements that should be included are:

- the name of the drug to which resistance is being tested (with the abbreviation)
- the target (could be called gene, locus, region or target)
- the method (name of test, description of test, e.g. pyrosequencing, Sanger sequencing)
- the result (Mutation Detected, include the nucleotide change and three letter amino acid change and numbering, as applicable)
- the interpretation

The table below is not inclusive of all potential molecular markers of drug resistance, but provides information for the most likely scenarios. This document will be updated as new markers are identified and used for molecular detection of drug resistance.

Interpretive comments will vary among laboratories. Comments are intended to provide supporting information including limitations and recommendations, to aid in clinical decision making and follow-up testing.

Regardless of result, the following additional interpretive comment should also be included: “Results must be interpreted in the context of other molecular or growth-based drug susceptibility test results and clinical history.”

Table 2: Suggested Reporting Language for Molecular Detection of Mutations Associated with Rifampin and Isoniazid^a

Drug	Target	Result	Interpretation	Additional Comments ^b
Rifampin (RIF)	<i>rpoB</i> gene	No mutation detected	Probably rifampin susceptible	Lack of mutation is indicative of probable susceptibility. XX% of rifampin resistant isolates have a mutation in <i>rpoB</i> . ^c
Rifampin (RIF)	<i>rpoB</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and three letter amino acid code change] (e.g. TCG>TTG; Ser 531Leu)	Rifampin resistant	Mutation confers RIF resistance. XX% of isolates with this mutation are rifampin resistant. ^c
Rifampin (RIF)	<i>rpoB</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and three letter amino acid code change] (e.g. CTG>CCG; Leu533Pro)	Clinical significance is unknown	Isolates with this mutation may test susceptible to rifampin by growth-based techniques. However, based on published reports, mutation may increase the risk of treatment failure or relapse.
Rifampin (RIF)	<i>rpoB</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and three letter amino acid code change].	Clinical significance is unknown	This is a novel mutation. Growth-based DST to follow. (1) This mutation has been detected previously by our laboratory at least once (2) This mutation has not been detected previously by our laboratory (3) The mutation has previously been reported in the literature [include reference]
Rifampin (RIF)	<i>rpoB</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and three letter amino acid code change] (e.g.TTC>TTT; Phe514Phe)	Probably rifampin susceptible	This silent mutation (no amino acid change) is not known to be associated with rifampin resistance.
Rifampin (RIF)	<i>rpoB</i> gene	No Amplification	Cannot provide information for rifampin due to lack of sequence data	An additional sample may be obtained and retested, if clinically indicated. Refer for growth-based DST.
Isoniazid (INH)	<i>katG</i> gene	No mutation detected	Probably isoniazid susceptible	Lack of mutation is indicative of susceptibility. XX% of isoniazid resistant isolates have a mutation in <i>katG</i> . ^c
	<i>inhA</i> promoter and gene	No mutation detected		
Isoniazid (INH)	<i>katG</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and 3 letter amino acid code change] (e.g. AGC>ACC; Ser315Thr)	Isoniazid resistant	Mutation confers INH resistance. XX% of isolates with this mutation in <i>katG</i> are isoniazid resistant. ^c
	<i>inhA</i> promoter and gene	No mutation detected		
Isoniazid (INH)	<i>katG</i> gene	No mutation detected	Isoniazid resistant	<i>inhA</i> mutations associated with resistance may confer low level INH resistance that correlates with growth-based DST resistance to the equivalent critical concentration of 0.2 ug/ml and susceptibility to 1.0 ug/ml of INH. ^d
	<i>inhA</i> promoter and gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and 3 letter amino acid code change if applicable] (e.g. C(-15)T)		
Isoniazid (INH)	<i>katG</i> gene	Mutation Detected [include mutation with MTBC numbering, nucleotide change and three letter amino acid code change]	Clinical significance is unknown	This is a novel mutation. Growth-based DST to follow. (1) This mutation has been detected previously by our laboratory at least once; (2) This mutation has not been detected previously by our laboratory; (3) The mutation has previously been reported in the literature [include Reference].
	<i>inhA</i> promoter and gene	No mutation detected		
Isoniazid (INH)	<i>katG</i> gene	No amplification	Cannot provide information for Isoniazid resistance due to lack of sequence data	An additional sample may be obtained and retested if clinically indicated. Refer for growth-based DST.
	<i>inhA</i> promoter and gene	No mutation detected		
Isoniazid (INH)	<i>katG</i> gene	No mutation detected	Cannot provide information for Isoniazid resistance due to lack of sequence data	An additional sample may be obtained and retested. Refer for growth-based DST.
	<i>inhA</i> promoter and gene	No amplification		

a. Recipients of these results are encouraged to seek expert consultation from the [State TB Control Program](#) and/or the [Regional Training Medical Consultation Center \(RTMCC\)](#) for their use for patient management.

b. The additional comments in this table can also be used to provide context or further information to the provider or other persons receiving the report. Some of the additional comments include multiple options (parenthetical numbers) depending on the scenario. Laboratories can choose the most appropriate comment(s) to include on their reports to accurately reflect their situation.

c. Data regarding the percentage of isolates that contain a mutation in a given gene target that confer resistance should be from a substantial data set (with regards to the number of isolate and appropriate geographic considerations) and could include results from one's own laboratory or published literature.

d. These are the concentrations for agar proportion. Please use the concentrations that are reported for the method you are using.

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1. CLSI. Molecular Diagnostic Methods for Infectious Diseases. 3rd. Ed. CLSI Report MM03. (ISBN 1-56238-997-1 [Print]; ISBN 1-56238-998-X [Electronic]). Clinical and Laboratory Standards Institute, 950 West Valley Road, Suite 2500, Wayne, Pennsylvania 19087 USA 2015.
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Resources

NTCA and APHL. Consensus statement on the use of the Cepheid Xpert MTB/RIF® assay in making decisions to discontinue airborne infection isolation in healthcare settings. April 2016. Available at: https://www.aphl.org/programs/infectious_disease/tuberculosis/Documents/NTCA_APHL_GeneXpert%20Consensus%20Statement_Final.pdf

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Regional Training Medical Consultation Centers (RTMCC): Available at: <https://www.cdc.gov/tb/education/rtmc/default.htm>

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