Test Definition: MTBT

MTB complex PCR, FFPE

Overview

Useful For

Preferred method for rapid detection of *Mycobacterium tuberculosis* complex DNA in formalin-fixed, paraffin-embedded tissue specimens

Detection of *M tuberculosis* complex

The PCR assay is **not intended for** the detection of latent tuberculosis and **must not be used** as a substitute for tests intended for detection of latent tuberculosis such as the tuberculin skin test (TST/PPD) or an interferon gamma release assay (IGRA).

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Tissue, Paraffin

Necessary Information

Specimen source is required.

Specimen Required

The high sensitivity of amplification by PCR requires the specimen to be processed in an environment in which contamination of the specimen by *Mycobacterium tuberculosis* DNA is unlikely.

Preferred Paraffin-embedded tissue block:

Supplies: Tissue Block Container (T553)

Specimen Type: Formalin-fixed, paraffin-embedded tissue block (FFPE)

Sources: Body tissue

Container/Tube: Tissue block

Collection Instructions: Submit a formalin-fixed, paraffin-embedded tissue block to be cut and returned.

Acceptable Paraffin-embedded tissue block:

Specimen Type: Formalin-fixed, paraffin-embedded tissue block (FFPE)

Sources: Body tissue

Container/Tube: Sterile container for each individual cut section (scroll).

Collection Instructions: Perform microtomy and prepare five separate 10-micron sections. **Each section (scroll) must be placed in a separate sterile container for submission.**

Specimen Minimum Volume

See Specimen Required.

Reject Due To

Other	Any non-FFPE tissue blocks, FFPE slides, FFPE body fluids

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Tissue, Paraffin	Ambient (preferred)		
	Refrigerated		

Clinical and Interpretive

Clinical Information

Each year, *Mycobacterium tuberculosis* accounts for approximately 1.4 million deaths and is responsible for 9 million newly diagnosed cases of tuberculosis worldwide. *M tuberculosis* is spread from person-to-person via respiratory transmission, and has the potential to become resistant to many or all of the antibiotics currently used if antimycobacterial treatment is not promptly initiated. Therefore, rapid and accurate detection of *M tuberculosis* in patient specimens is of clinical and public health importance.

Conventional culture methods can generally detect *M tuberculosis* in 2 to 3 weeks, although up to 8 weeks of incubation may be required in some instances. Developed at Mayo Clinic, this rapid PCR assay detects *M tuberculosis* complex DNA directly from respiratory specimens and other specimens without waiting for growth in culture and, therefore, the results are available the same day the specimen is received in the laboratory. A mycobacterial culture should always be performed in addition to the PCR assay. This assay is rapid but the culture has increased sensitivity over it. The PCR assay targets a unique sequence within the *katG* gene, which is present in members of the *M tuberculosis* complex. In addition, the assay can detect genotypic resistance to isoniazid mediated by variants in the *katG* target, when present.

Reference Values

Not applicable

Interpretation

A positive result indicates the presence of *Mycobacterium tuberculosis* complex DNA. Members of the *M tuberculosis* complex detected by this assay include *M tuberculosis, M bovis, M bovis* bacillus Calmette-Guerin (BCG*), M africanum, M canettii*, and *M microti.* The other species within the *M tuberculosis* complex (eg, *M caprae, M pinnipedii, and M mungi*) should, in theory, be detected using the primer and probe sequences in this assay, but they have not been tested at this time. This assay method does not distinguish between the species of the *M tuberculosis* complex.

A negative result indicates the absence of detectable *M tuberculosis* complex DNA.

Isoniazid (INH) resistance mediated through a *katG* variant will be reported when observed but lack of a *katG* variant does not imply that the isolate is susceptible to INH. There are other genetic loci in addition to *katG* that can contribute to resistance for this drug.

An inhibition result indicates that inhibitors are present in the specimen that could prevent the detection of *M tuberculosis* DNA. A new specimen can be resubmitted under a new order, if desired.

Cautions

This rapid PCR assay detects *Mycobacterium tuberculosis* complex nucleic acid and, therefore, does not distinguish between viable, disease-related organisms and nucleic acid persisting from prior infection. Test results should be correlated with patient symptoms and clinical presentation before a definitive diagnosis is made.

A negative result does not rule out the presence of *M tuberculosis* complex or active disease because the organism may be present at levels below the limit of detection for this assay.

This test has not been studied for use with specimens from patients being treated with antituberculous agents and, therefore, should not be used to determine bacteriologic cure or to monitor response to therapy. It is not known how long the PCR assay can remain positive following treatment for *M tuberculosis*.

The sensitivity of this test from formalin-fixed, paraffin-embedded tissue is approximately 63%; therefore, testing of additional specimens should be considered if the result from the first specimen is negative.

Supportive Data

The analytical specificity of the assay was determined using Basic Local Alignment Search Tool (BLAST) analysis of the National Center for Biotechnology Information (NCBI) GenBank database and no sequences were detected that would interfere with the LightCycler PCR assay. Further, the assay was tested using a panel of 104 respiratory pathogens (bacteria and viruses) that were extracted and subjected to the LightCycler PCR assay. As predicted, only *Mycobacterium tuberculosis* complex was detected from this panel. In addition, nearly 100 species of nontuberculous mycobacteria were evaluated using this PCR assay and there was no cross-reactivity detected. The analytical sensitivity of the assay was determined to be 10 target copies/microliter using a dilution series of *Mycobacterium tuberculosis* spiked into respiratory specimens in triplicate.

The inhibition rate from formalin-fixed, paraffin-embedded tissue was 37% (19 of 30 spiked specimens were positive).

Two melt peaks can be produced during this assay. A melt peak at a Tm 64.0 degrees C + or - 2.5 degrees C can correspond to either isoniazid-susceptible or isoniazid-resistant *M tuberculosis* and, therefore, no indication of isoniazid susceptibility is provided for these isolates. However, an isolate with a melt peak occurring at a Tm of 58.0 degrees C + or - 2.5 degrees C correlated with isoniazid resistance determined using a broth reference method in 100% (26/26) of isolates tested. Isolates with a peak at a Tm of 58.0 degrees C + or - 2.5 degrees C are reported as "Positive, probable isoniazid resistance detected." The PCR result is available 7 to 14 days prior to the broth method and, therefore, may be helpful in selecting appropriate antibiotic therapy for these patients. Confirmation of isoniazid resistance must be ordered if the isolate grows in culture.

Clinical Reference

1. Iseman MD: A clinician's guide to tuberculosis. Second edition. Philadelphia, PA. Lippincott Williams and Wilkins, 2013

2. Centers for Disease Control and Prevention: Treatment of Tuberculosis, American Thoracic Society, CDC, and Infectious Diseases Society of America. MMWR Morb Mortal Weekly Rep 2003;52(No. RR-11):1-88

Performance

Method Description

Following specimen processing, genomic DNA is extracted and the purified genomic DNA is placed on the LightCycler instrument, which amplifies and monitors, by fluorescence, the development of target nucleotide sequences after each PCR cycle. A specific target sequence from a portion of the *kat*G gene from *Mycobacterium tuberculosis* complex is amplified and the resulting segment is detected by melt-curve analysis using sequence-specific fluorescence resonance energy transfer hybridization probes. The LightCycler PCR assay is a closed PCR system that greatly reduces the potential for false-positive results due to specimen cross-contamination as compared with traditional open-system PCR or other amplification methods like transcription-mediated amplification.(Unpublished Mayo method)

PDF Report

No

Day(s) and Time(s) Test Performed

Monday through Friday

Analytic Time

5 days

Maximum Laboratory Time

7 days

Specimen Retention Time

7 days; after which time the block will be returned to the client

Performing Laboratory Location

Rochester

Fees and Codes

Fees

- Authorized users can sign in to Test Prices for detailed fee information.
- Clients without access to Test Prices can contact Customer Service 24 hours a day, seven days a week.
- Prospective clients should contact their Regional Manager. For assistance, contact Customer Service.

Test Classification

This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. This test has not been cleared or approved by the U.S. Food and Drug Administration.

CPT Code Information

87556-*Mycobacterium tuberculosis*, complex, molecular detection, PCR, Paraffin

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
MTBT	MTB complex PCR, FFPE	38379-4

Test Definition: MTBT

MTB complex PCR, FFPE

Result ID	Test Result Name	Result LOINC Value
SRCTB	MTB Complex PCR, FFPE, Source	31208-2
TBRR	MTB Complex PCR, FFPE, Result	38379-4