Test Definition: MTBRP

M tuberculosis Complex PCR

Overview

Useful For

Rapid detection of *Mycobacterium tuberculosis* complex DNA, preferred method

Detection of *M tuberculosis*, when used in conjunction with mycobacterial culture

This test should **not be used** to determine bacteriologic cure or to monitor response to therapy.

This test 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).

Reflex Tests

Test ID	Reporting Name	Available Separately	Always Performed
TBT	Concentration, Mycobacteria	No, (Bill Only)	No

Testing Algorithm

When this test is ordered, the reflex test may be performed and charged.

See Meningitis/Encephalitis Panel Algorithm in Special Instructions.

Special Instructions

- Meningitis/Encephalitis Panel Algorithm

Method Name

Real-Time Polymerase Chain Reaction (PCR)

NY State Available

Yes

Specimen

Specimen Type

Varies

Additional Testing Requirements

This test should always be performed in conjunction with mycobacterial culture.

Shipping Instructions

Specimen must arrive within 7 days of collection; specimen >7 days will be rejected.

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.

If a single specimen is being shared between mycobacteria culture, acid-fast smear, and/or *M tuberculosis* PCR, a minimum volume of 2 mL for body fluid, 3 mL for respiratory specimen, or a pea-sized piece of tissue should be obtained. Specimen volumes less than indicated may decrease sensitivity of testing. If insufficient volume is submitted, test or tests will be canceled.

Preferred Specimens: Body fluid, cerebrospinal fluid (CSF), ocular fluid, respiratory (eg, bronchoalveolar lavage [BAL], bronchial washing, sputum), feces, fresh tissue, bone, bone marrow, or urine

Acceptable Specimens: If no fresh specimen is available, digested respiratory specimens treated with N-acetyl-L-cysteine/sodium hydroxide (NALC/NaOH) are acceptable (eg, BAL, bronchial washing, respiratory fluid, sputum, or tracheal secretion), as are NALC/NaOH-treated gastric washings.

Submit only 1 of the following specimens:

Specimen Type: Body fluid

Sources: Body, bone marrow aspirate, ocular, or CSF

Container/Tube: Sterile container

Specimen Volume: 1 mL

Additional Information: Only fresh, non-NALC/NaOH-digested body fluid is acceptable.

Specimen Type: Gastric washing

Container/Tube: Sterile container

Specimen Volume: 2 mL

Collection Instructions: Neutralize specimenwithin 4 hours of collection with 20 mg of sodium carbonate per 2 mL of gastric washing.

Specimen Type: Respiratory

Sources: BAL, bronchial washing, or sputum

Container/Tube: Sterile container

Specimen Volume:1 mL if only PCR ordered or 3 mL if PCR ordered with smear and culture

Specimen Type: Feces

Container/Tube: Sterile container

Specimen Volume: 5-10 g

Additional Information: Only fresh, non-NALC/NaOH-digested fecal specimens are acceptable.

Specimen Type: Tissue

Sources: Fresh tissue, bone, or bone marrow biopsy

Container/Tube: Sterile container

Specimen Volume: 5-10 mm

Collection Instructions: Keep moist with sterile water or sterile saline

Additional Information: Only fresh, non-NALC/NaOH-digested tissue is acceptable.

Specimen Type: Urine

Container/Tube: Sterile container

Specimen Volume: 1mL

Collection Instructions: Collect a random urine specimen.

Acceptable

Specimen Type: NALC/NaOH-digested respiratory specimens

Sources: Lavage fluid, bronchial washing, gastric washing, respiratory fluid, sputum, or tracheal secretion

Container/Tube: Sterile container

Specimen Volume: 2 mL

Collection Instructions:

1. Submit digested specimen treated with NALC/NaOH.

2. Clearly indicate on container and order form that specimen is a digested specimen.

Forms

If not ordering electronically, complete, print, and send a Microbiology Test Request (T244) with the specimen.

Specimen Minimum Volume

Body fluid: 0.5 mL
Respiratory specimen-nondigested: 0.5 mL
Fresh tissue or bone: 5 mm
NALC-NaOH-digested specimen: 1 mL
Gastric washing: 1 mL
Stool: 5 g
Urine: 0.5 mL

Reject Due To

Other	Blood Specimen in anaerobe vial or viral transport medium (including but not limited to M4, M5, BD viral transport media, thioglycolate broth) Swabs Tissues in formalin fluid

Specimen Stability Information

Specimen Type	Temperature	Time	Special Container
Varies	Refrigerated (preferred)	7 days	
	Frozen	7 days	

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. The PCR assay is rapid but the culture has increased sensitivity over the PCR assay. 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 mutations 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.* 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. 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.

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 with stool specimens is 80% and testing of additional stool 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 *M tuberculosis* spiked into respiratory specimens in triplicate.

The sensitivity and specificity of the assay for detection of *M tuberculosis* complex verses culture was found to be 100% and 100% respectively for 26 *M tuberculosis*-positive cultures and 266 *M tuberculosis*-negative cultures. The PCR inhibition rate of the assay was determined to be 0% by spiking 100 negative extracted respiratory specimens with *M tuberculosis* at 100 targets/microliter. The PCR assay was able to detect 100% of specimens spiked at the limit of detection for bronchoalveolar lavage (BAL) fluid, muscle/skin tissue, organ tissues, bone, cerebrospinal fluid (CSF), and urine. One of 30 (3%) of spiked respiratory tissues was inhibited and 3 of 30 (10%) of spiked sterile body fluids other than CSF were inhibited. Not surprising increased inhibition was seen in stool (24 of 30 spiked specimens were positive) and formalin-fixed, paraffin-embedded tissue (19 of 30 spiked specimens were positive).

Method comparison of the LightCycler PCR assay versus mycobacterial culture was done using 192 respiratory specimens. The results are shown in Table 1.

Table 1. Results for the LightCycler PCR assay versus mycobacterial culture for 192 respiratory specimens.

		Culture positive for *Mycobacterium tuberculosis* complex			
		+	**-**	**Sensitivity**	**Specificity**
LightCycler PCR	**+**	33	1	87%	99%
	-	5	153		

Table 2 provides a comparison of the LightCycler PCR assay versus the GEN-PROBE Mycobacterium tuberculosis Direct (MTD) assay, performed using 542 respiratory specimens (226 BAL fluids, bronchial washings and lung washings plus 316 sputa, induced sputa, and tracheal secretions). The kappa coefficient of 0.96 indicates excellent

agreement between the 2 methods.

Table 2. Clinical sensitivity of the LightCycler PCR versus MTD for respiratory specimens.

Assay		MTD +	MTD -	Agreement (%)	kappa coefficient
LightCycler PCR	+	49	1	538/542 (99.3%)	0.96
	-	3	489		

Two melt peaks can be produced during this assay. A melt peak at a temperature of 64.0 degrees C + or - 2.5 degrees C can correspond to either isoniazid-susceptible or isonazid-resistant *M tuberculosis* and, therefore, no indication of isoniazid susceptibility is provided for these isolates. However, an isolate with melt peak occurring at a temperature 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 temperature 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 isonaizid resistance must be done using a phenotypic method if the isolate grows in culture.

Clinical Reference

1. Iseman MD: A clinicianÃ¢â‚¬â„¢s guide to tuberculosis. Philadelphia, PA. Lippincott Williams and Wilkins, 2000

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 digestion and decontamination using N-acetyl cysteine and sodium hydroxide, genomic DNA is extracted using the MagNA Pure Compact (Roche Applied Sciences) extraction platform. 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) Performed

Monday through Sunday

Report Available

1 to 3 days

Specimen Retention Time

7 days

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 US Food and Drug Administration.

CPT Code Information

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

87015-Mycobacteria culture, concentration (if appropriate)

LOINC® Information

Test ID	Test Order Name	Order LOINC Value
MTBRP	M tuberculosis Complex PCR	38379-4

Result ID	Test Result Name	Result LOINC Value
SRC62	MTB Complex PCR, Specimen Source	31208-2
56044	MTB Complex PCR, Result	38379-4