

Test Definition: MPRP

Mycoplasma (Mycoplasma) pneumoniae
with Macrolide Resistance Reflex, Molecular
Detection, PCR, Varies

Overview

Useful For

Diagnosing infections due to *Mycoplasma (Mycoplasma) pneumoniae*

Assessing macrolide susceptibility

Reflex Tests

Test Id	Reporting Name	Available Separately	Always Performed
RPMPM	M. pneumoniae Macrolide Resist PCR	Yes	No

Testing Algorithm

If positive, *Mycoplasma pneumoniae* macrolide resistance will be performed at an additional charge.

Method Name

Rapid Polymerase Chain Reaction (PCR) using Light Cycler and Fluorescent Resonance Energy Transfer (FRET)

NY State Available

Yes

Specimen

Specimen Type

Varies

Necessary Information

Specimen source is required.

Specimen Required

The high sensitivity of amplification by polymerase chain reaction requires the specimen to be processed in an environment in which contamination of the specimen by *Mycoplasma (Mycoplasma) pneumoniae* DNA is unlikely.

Submit only 1 of the following specimens:

Specimen Type: Swab

Supplies:

-Culturette (BBL Culture Swab) (T092)

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- BD E-Swab (T853)
- Nasopharyngeal Swab (Nylon Mini-Tip Swab) (T861)
- Culture Swab-Liquid Stuarts/Single Swab (NP Swab) (T515)
- M4-RT (T605)

Sources: Throat, nasal, or nasopharyngeal

Container/Tube:

Preferred: Culture swab transport system (Dacron or rayon swab with aluminum or plastic shaft with either Stuart or Amies liquid medium)

Acceptable: Culture transport swab (Stuart's media) or place swab in M4, M4-RT, M5, M6, universal transport media, or ESwab

Specimen Volume: Swab

Collection Instructions:

1. Collect specimen by swabbing back and forth over mucosa surface to maximize recovery of cells.
2. Place swab back into swab cylinder.

Specimen Type: Fluid

Sources: Pleural, pericardial, cerebrospinal

Container/Tube: Sterile container

Specimen Volume: 0.5 mL

Specimen Type: Respiratory

Sources: Bronchial washing, bronchoalveolar lavage, tracheal secretions, sputum

Container/Tube: Sterile container

Specimen Volume: 1 mL

Forms

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

Specimen Minimum Volume

Respiratory specimen: 0.5 mL

Other specimen types: See Specimen Required

Reject Due To

Cotton or calcium alginate-tipped swab, wooden shaft swab, transport swab containing gel or charcoal Port-a-Cul tube	Reject
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Anaerobic fluid
vials
Dry swab (no
pledget or
sponge)
Respiratory
fluid
specimens
placed in VTM
or placed on a
swab and then
into VTM
(M4-RT, M4, or
M5)
Body fluid
specimens
placed in VTM
or placed on a
swab and then
in VTM
(M4-RT, M4, or
M5)

Specimen Stability Information

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

Clinical & Interpretive

Clinical Information

Mycoplasma (Mycoplasma) pneumoniae is a small bacterium transmitted via organism-containing droplets. It is a cause of upper respiratory infection, pharyngitis, and tracheobronchitis, particularly in children, and has been associated with approximately 20% of cases of community acquired pneumonia.(1) Central nervous system and cardiac manifestations are some of the extrapulmonary complications of infections due to *M pneumoniae*. The disease is usually self-limited although severe disease may occur, including in patients who are immunocompromised.(2)

Identification of *M pneumoniae* by culture-based methods is time consuming and insensitive. Serologic assays have drawbacks; the development of IgM antibodies takes approximately 1 week, and the IgM response in adults may be variable or may be decreased in immunosuppressed individuals.(3,4) Confirmation of the disease may be dependent on

the observation of a 4-fold rise in IgG antibody titers between acute and convalescent specimens, only after several weeks following the initial onset of illness, only providing clinical application for retrospective testing and not individual patient care.(4) Real-time polymerase chain reaction (PCR) testing offers a rapid and sensitive option for detection of *M pneumoniae* DNA from clinical specimens.(5)

Macrolide resistance in *M pneumoniae* is increasingly reported. In a study performed at Mayo Clinic, 10% of *M pneumoniae* detections were associated with macrolide resistance.(6) Real-time PCR testing can be used to assess for common mutations associated with macrolide resistance in *M pneumoniae*.

Reference Values

Negative

Interpretation

A positive result indicates the presence of *Mycoplasma (Mycoplasma) pneumoniae*. If detected, common mutations associated with macrolide resistance in *M pneumoniae* may be assessed.

A negative result does not rule out the presence of *M pneumoniae* and may be due to the presence of inhibitors within the specimen matrix or the presence of target DNA below the limit of detection of the assay.

Cautions

This assay should only be used for testing of respiratory tract specimens (throat swabs, nasopharyngeal swabs, tracheal secretions, sputum, and bronchoalveolar lavage fluid) and pleural/chest fluid, pericardial fluid, and cerebrospinal fluid.

Supportive Data

Accuracy:

The assay was validated in a blinded manner using 30 *Mycoplasma (Mycoplasma) pneumoniae*-positive specimens received from a reference lab and 6 negative specimens. The *M pneumoniae* polymerase chain reaction (PCR) test had 100% sensitivity and specificity when compared to the Focus Diagnostics *M pneumoniae* primer pair PCR assay. Whole organism spiking studies (near the limit of detection of the assay) were also performed using the following specimens: bronchoalveolar lavage/bronchial wash, nasopharyngeal and throat swabs, sputum, pericardial/pleural fluid, and cerebrospinal fluid. These specimens were confirmed as being negative for *M pneumoniae* prior to spiking. The sensitivity and specificity of the spiked specimens combined for all the matrices were 99% (154/155) and 100% (57/57), respectively.

Limit of detection:

The limit of detection of the assay is less than 5 target copies/mL for all validated specimen types.

Analytical specificity:

The assay was tested against a panel of 45 organisms consisting of bacteria and viruses representing normal respiratory flora and/or respiratory pathogens. There was no cross reactivity among these organisms, which included 16 other species of *Mycoplasma (Mycoplasma)*.

Clinical Reference

1. Waites KB, Taylor-Robinson D: Mycoplasma and Ureaplasma. In: Versalovic J, Carroll K, Funke G, et al, eds. Manual of

Clinical Microbiology. ASM Press; 2011:970-985

2. Jensen JS, Heilmann C, Valerius NH. Mycoplasma pneumoniae infection in a child with AIDS. Clin Infect Dis. 1994;19(1):207

3. Daxboeck F, Krause R, Wenisch C. Laboratory diagnosis of Mycoplasma pneumoniae infection. Clin Microbiol Infect. 2003;9(4):263-273

4. Waites KB, Talkington DF. Mycoplasma pneumoniae and its role as a human pathogen. Clin Microbiol Rev. 2004;17(4):697-728

5. Schmitt BH, Sloan LM, Patel R. Real-time PCR detection of Mycoplasma pneumoniae in respiratory specimens. Diagn Microbiol Infect Dis. 2013;77(3):202-205

6. Rothstein TE, Cunningham SA, Rieke RA, Mainella JM, Mutchler MM, Patel R. Macrolide resistance in Mycoplasma pneumoniae, Midwestern United States, 2014 to 2021. Antimicrob Agents Chemother. 2022;66(4):e0243221

Performance

Method Description

Throat swabs, nasopharyngeal swabs, sputum, bronchoalveolar lavage fluid, pericardial/pleural/chest fluid, and cerebrospinal fluid specimens are processed according to specimen type. Nucleic acid is extracted by the MagNA Pure 96 automated instrument (Roche Applied Science). A specific target sequence from *Mycoplasma (Mycoplasmoides) pneumoniae* is targeted by primers and fluorescence resonance energy transfer hybridization probes. The LightCycler 480 II instrument (Roche Applied Science) amplifies and monitors the development of target nucleic acid sequences after the annealing step during polymerase chain reaction (PCR) cycling. Detection of the *M pneumoniae* target is performed through melting curve analysis using the LightCycler software. (Schmitt BH, Sloan LM, Patel R. Real-time PCR detection of *Mycoplasma pneumoniae* in respiratory specimens. Diagn Microbiol Infect Dis. 2013 Nov;77[3]:202-205)

If *M pneumoniae* is detected, a reflexive PCR is performed to assess the 23S ribosomal RNA gene region of *M pneumoniae* and predict macrolide resistance based on the most common, high-level point mutations at positions 2064 and 2063 via melting curve analysis. While the wildtype genotype will display a stable melting temperature, the designed primer and probe combinations will be highly sensitive to single nucleotide mutations resulting in a cooler (left shift) melting temperature value. (Rothstein TE, Cunningham SA, Rieke RA, Mainella JM, Mutchler MM, Patel R. Macrolide resistance in Mycoplasma pneumoniae, Midwestern United States, 2014 to 2021. Antimicrob Agents Chemother. 2022 Apr 19;66[4]:e0243221)

PDF Report

No

Day(s) Performed

Monday through Sunday

Report Available

3 to 4 days

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Specimen Retention Time

7 days

Performing Laboratory Location

Mayo Clinic Laboratories - Rochester Main Campus

Fees & 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 account representative. 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. It has not been cleared or approved by the US Food and Drug Administration.

CPT Code Information

87581

LOINC® Information

Test ID	Test Order Name	Order LOINC® Value
MPRP	M pneumoniae PCR + Macrolide Reflex	29257-3

Result ID	Test Result Name	Result LOINC® Value
SRCMP	Specimen source	31208-2
62394	M. pneumoniae PCR	29257-3