

## Test Definition: RPMPM

*Mycoplasma (Mycoplasmoides) pneumoniae*  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

---

### Overview

#### Useful For

Predicting macrolide susceptibility in *Mycoplasma (Mycoplasmoides) pneumoniae*

#### Method Name

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

#### NY State Available

Yes

### Specimen

#### Specimen Type

Varies

#### Ordering Guidance

This test should only be ordered on specimens that have tested positive for *Mycoplasma (Mycoplasmoides) pneumoniae*. This assay predicts *M pneumoniae* macrolide (Azithromycin) resistance only.

For detection of *M pneumoniae* prior to macrolide resistance testing, order MPRP / *Mycoplasma (Mycoplasmoides) pneumoniae* with Macrolide Resistance Reflex, Molecular Detection, PCR, Varies.

#### Necessary Information

**Specimen source is required; include the specific anatomic source.**

#### 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 (Mycoplasmoides) pneumoniae* DNA is unlikely.

**Submit only 1 of the following specimens:**

**Specimen Type:** Swab

#### Supplies:

- Culturette (BBL Culture Swab) (T092)
- BD E-swab (T853)
- Culture Swab-Liquid Stuarts/Single Swab (NP Swab) (T515)
- M4-RT (T605)

**Sources:** Throat, nasal, or nasopharyngeal

## Test Definition: RPMPM

Mycoplasma (Mycoplasma) pneumoniae  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

### 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: 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 Anaerobic fluid vials Dry swab (no pledget or sponge)	Reject
---	--------

## Test Definition: RPMPM

Mycoplasma (*Mycoplasma*) pneumoniae  
 Macrolide (Azithromycin) Resistance  
 Prediction, Molecular Detection, PCR, Varies

Respiratory fluid specimens placed in viral transport medium (VTM) or placed on a swab and then into VTM (M4-RT, M4, or M5) Body fluid specimens placed in viral transport medium (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)

Macrolide resistance in *M pneumoniae* has steadily increased since the early 2000s. Reports suggest over 90% of *M pneumoniae* isolates are now macrolide resistant in areas of Japan and China, with macrolide resistance also noted in other countries.(3) Macrolides are a common treatment for respiratory tract infections and *M pneumoniae*. Resistance in *M pneumoniae* typically corresponds to single point mutations in the 23S ribosomal RNA of the 50S bacterial

## Test Definition: RPMPM

*Mycoplasma (Mycoplasmoides) pneumoniae*  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

---

ribosomal subunit. Among the reported point mutations, mutations at positions 2064 and 2063 are the most common and confer to high-level macrolide resistance.(3) In a study performed at Mayo Clinic, 10% of *M pneumoniae* detections were associated with macrolide resistance.(4)

Culture, serologic testing, and molecular-based techniques can be used to detect *M pneumoniae* infection. While detection of macrolide resistance may be determined through culture methods (with antimicrobial susceptibility testing), it is impractical due to the organism's slow and fastidious growth requirements. Real-time polymerase chain reaction testing can be used to assess for common mutations associated with macrolide resistance in *M pneumoniae*.

### Reference Values

Not Predicted

### Interpretation

A macrolide resistance predicted or not predicted result indicates the presence of *Mycoplasma (Mycoplasmoides) pneumoniae* 23S ribosomal RNA (rRNA) gene and indicates whether one of the 2 most common 23S rRNA gene single nucleotide variations (A2064G and A2063G) associated with high-level macrolide resistance is predicted.

An "unable to assess" result for *M pneumoniae* macrolide resistance indicates the absence of detectable *M pneumoniae* 23S rRNA DNA but does not negate the presence of the organism and may occur due to inhibition of the polymerase chain reaction, sequence variability underlying primers or probes, or the presence of *M pneumoniae* 23S rRNA DNA in quantities less than 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 that has already tested positive for *Mycoplasma (Mycoplasmoides)pneumoniae* using a nucleic acid amplification test.

Test results should be used as an aid in the diagnosis. The single assay should not be used as the only criterion to form a treatment decision; results of this test should be correlated with clinical presentation and results of other laboratory tests. A negative result does not negate the presence of the organism or active disease.

Rarely encountered *Mycoplasma* species may be detected with this assay when present at high concentrations, however this assay is intended to be used as reflex for previously identified *M pneumoniae* positive samples. Therefore, cross reactivity with other *Mycoplasma* species is not a major concern.

This assay examines the two most common 23S ribosomal RNA single point variants associated with high-level macrolide resistance. Other mechanisms of macrolide resistance are not assessed nor are mechanisms of resistance to non-macrolide antimicrobial agents.

### Supportive Data

Accuracy:

During laboratory verification studies, 129 respiratory specimens (86 respiratory swabs, 16 bronchoalveolar lavage, 1 pleural/pericardial, 25 sputum, and 1 cerebrospinal fluid) previously tested via Mayo Clinic's *Mycoplasma (Mycoplasmoides) pneumoniae* PCR test were reflexed for macrolide prediction. Upon reflex, this assay detected *M*

## Test Definition: RPMPM

*Mycoplasma (Mycoplasmoides) pneumoniae*  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

---

*pneumoniae* 23S ribosomal RNA (rRNA) DNA in 114/129 samples (89.4% overall percent agreement).

Assessment of macrolide resistance prediction was made by performing bidirectional Sanger sequencing on all macrolide (Azithromycin) resistant samples. All 103 samples with predicted macrolide susceptible *M pneumoniae*, demonstrated wildtype 23S rRNA gene sequence at positions 2063 and 2064. All 11 samples with predicted clarithromycin resistant *M pneumoniae*, demonstrated single nucleotide polymorphisms of A2063G or A2064G with the *M pneumoniae* 23S rRNA gene.

### Limit of detection:

The limit of detection of the assay is less than 10 target copies/mcL for all validated specimen types and gene targets.

### Analytical specificity:

The assay was tested against a panel of 87 organisms consisting of bacteria and viruses representing normal respiratory microbiota and/or respiratory pathogens (including 26 Mollicute species). Among the 23 other species of *Mycoplasma (Mycoplasmoides)* tested, there was cross-reactivity noted among *Mycoplasma (Mycoplasmoides) testudinis* (likely not found in humans and has not been reported to cause disease in humans) and *Mycoplasma (Mycoplasmoides) pirum*. This assay is intended to be used as reflex for previously identified *M pneumoniae* positive samples. Therefore, this cross reactivity with other *Mycoplasma (Mycoplasmoides)* species is not a major concern.

### 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. Waites KB, Xiao L, Liu Y, Balish MF, Atkinson TP. *Mycoplasma pneumoniae* from the Respiratory Tract and Beyond. *Clin Microbiol Rev*. 2017;30(3):747-809
4. 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
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

### Performance

#### Method Description

When *Mycoplasma (Mycoplasmoides) pneumoniae* is detected via previous nucleic acid amplification test, a reflexive polymerase chain reaction (PCR) is performed to assess the 23S ribosomal RNA (rRNA) 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. Note, samples deemed positive outside of Mayo Clinic Laboratories may be processed according to specimen type prior to PCR testing. This includes extraction by the MagNA Pure 96 automated instrument (Roche Applied Science).

## Test Definition: RPMPM

Mycoplasma (Mycoplasmoides) pneumoniae  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

---

A specific target sequence of the 23S rRNA gene from *M 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 PCR cycling. Detection and prediction of the *M pneumoniae* target is performed through melting curve analysis using the LightCycler software. While the wild-type 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. PCR inhibition is monitored through use of a recovery template. (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; 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)

### PDF Report

No

### Day(s) Performed

Monday through Sunday

### Report Available

3 to 4 days

### 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

87798

### LOINC® Information

## Test Definition: RPMPM

Mycoplasma (Mycoplasma) pneumoniae  
Macrolide (Azithromycin) Resistance  
Prediction, Molecular Detection, PCR, Varies

Test ID	Test Order Name	Order LOINC® Value
RPMPM	M. pneumoniae Macrolide Resist PCR	6483-2

Result ID	Test Result Name	Result LOINC® Value
SRCMP	Specimen source	31208-2
619928	M. pneumoniae Macrolide Resistance	6483-2