Mycoplasma Pneumoniae IgG/IgM Antibody Test

INTENDED USE
Remel Mycoplasma Pneumoniae IgG/IgM Antibody Test is an enzyme-linked immunobinding assay for qualitative simultaneous detection of IgG/IgM antibodies to Mycoplasma pneumoniae in human serum or plasma (EDTA, citrate, or heparin).

SUMMARY AND EXPLANATION
*M. pneumoniae* is the most common cause of primary atypical pneumonia and febrile upper respiratory tract infections in the world. The involvement of other organ systems may occur with fatal life-threatening consequences. *M. pneumoniae* has a peak incidence in individuals 5 to 15 years of age and accounts for as much as 20% of all pneumonias observed among children and young adults. The incubation period is 2-3 weeks and infection occurs throughout the year; though it is seen more frequently in the fall and winter months. Antibiotics (i.e., tetracycline and erythromycin) are used to treat mycoplasmal pneumonia. Without treatment, the symptoms may decline but the organism persists. Frequently the inflammatory response recurs, occasionally with extrapulmonary complications. Other diagnostic tests have been used (i.e., complement fixation and cold agglutinin) but generally do not detect acute phase IgM antibody. The test for cold agglutinins is less useful since these antibodies develop in only about 20% of patients with mycoplasmal pneumonia and are also induced by several other diseases.

*M. pneumoniae* cross-reacting glycolipids are the predominant complement-fixation antigenic determinants responsible for inducing circulating antibody. A polysaccharide-protein fraction is purported to be the component stimulating cell-mediated immunity, and the cytdhesin protein is now used in enzyme-linked immunosorbent assays.

PRINCIPLE
Mycoplasma Pneumoniae IgG/IgM Antibody Test utilizes a permeable immunobinding membrane. Patient serum or plasma is reacted with *M. pneumoniae* antigen, immobilized on the membrane. Antibodies to *M. pneumoniae*, if present in the serum or plasma, bind to the antigen. Enzyme-labeled anti-human IgG/IgM added to the reaction site binds with IgG/IgM antibodies in the test specimen. Following addition of substrate/chromogen, a visible purple dot (of varying intensity) develops exhibiting particulate matter is not recommended as such specimens may cross-react with other antigens. Serum: Use serum prepared from freshly collected whole blood. Allow blood to fully clot before centrifuging. Plasma: Collect blood in a tube containing EDTA, heparin, or citrate anticoagulant. Promptly centrifuge the blood and separate the plasma from the cells.

TEST MODULES
1. Test Modules: Each test module consists of one test well and two reagent/procedure control wells spotted on a reaction membrane. Each well contains extracts of inactivated *M. pneumoniae* protein antigen (primarily cytdhesin protein).
2. Specimen Diluent (Reagent A): 1 vial containing phosphate buffer with stabilizers and 0.01% thimerosal
3. Conjugate (Reagent B): 1 vial containing horseradish peroxidase (HRP) labeled anti-human IgG and IgM with stabilizers and 0.01% thimerosal
4. Conjugate Rinse (Reagent C): 1 vial containing buffer solution
5. Color Developer (Reagent D): 1 vial containing substrate with stabilizers
6. Positive Control: 1 vial containing buffered human IgG and/or IgM antibodies to *M. pneumoniae* with stabilizers and 0.01% thimerosal
7. Negative Control: 1 vial containing buffered human serum devoid of antibodies to *M. pneumoniae* with stabilizers and 0.01% thimerosal
8. Micropipettes
9. Specimen Cups
10. Instructions for Use (IFU)

MATERIALS SUPPLIED
- Test Modules: 10 each
- Specimen Diluent (Reagent A): 5.0 ml
- Conjugate (Reagent B): 1.5 ml
- Conjugate Rinse (Reagent C): 3.0 ml
- Color Developer (Reagent D): 1.5 ml
- Positive Control: 0.5 ml
- Negative Control: 0.5 ml
- Specimen Cups: 10 each
- Micropipettes: 20 each

MATERIALS REQUIRED BUT NOT SUPPLIED
- (1) Centrifuge, (2) Timer, (3) Blood Collection Devices, (4) 0.22 µm Millex GV Millipore syringe filter or equivalent (required for frozen specimens).

SPECIMEN COLLECTION, STORAGE, AND TRANSPORT
Specimens should be collected and handled following recommended guidelines. Serum: Use serum prepared from freshly collected whole blood. Allow blood to fully clot before centrifuging. Plasma: Collect blood in a tube containing EDTA, heparin, or citrate anticoagulant. Promptly centrifuge the blood and separate the plasma from the cells. If testing cannot be performed on the day of collection, specimens may be stored at 2-8°C for up to 5 days. For longer storage, freeze specimens at below -20°C. Specimens which have been frozen must be filtered prior to testing. A 0.22 µm filter may be used after the dilution step (Procedure, 1.b.). Avoid repeated freezing and thawing of specimens. The use of serum or plasma that is grossly lipemic, contaminated with bacteria, or exhibits particulate matter is not recommended as such specimens may produce aberrant results.

PROCEDURE
- Allow reagents and specimens to equilibrate to room temperature prior to use.
- Mix reagents by inverting the vials several times. When dispensing reagents, hold vials vertically and dispense only free-falling drops.
- Do not touch test module membranes.
- Hold micropipettes vertically when dispensing specimens.

1. Dilute the specimen:
   a. Using a micropipette (provided) add 1 drop of serum or plasma to a specimen cup.
   b. Dispense six drops of Reagent A into cup containing the specimen.
      If specimen has been frozen, filter dilution before proceeding.
2. Prepare the Test Module:
   a. Using a fresh micropipette, dispense 1 drop of diluted specimen into the test well (TEST) of the test module.
   b. Dispense 1 drop of Negative Control into the negative (-) well.
   c. Dispense 1 drop of Positive Control into the positive (+) well.
3. Incubate the test module at room temperature for 1 minute.
4. Dispense 1 drop of Reagent A into each well. Allow the reagent to completely wick into the membrane.
5. Dispense 1 drop of Reagent B into each well. Allow the reagent to completely wick into the membrane.
6. Dispense 1 drop of Reagent C into each well. Allow the reagent to completely wick into the membrane (approximately 30 seconds).
7. Dispense 1 drop of Reagent D into each well.
8. Observe for the formation of a purple color at 2 to 5 minutes. Test-well reactions may range from no color to a very dark purple.
READING OF RESULTS

Positive Result - A purple color in the TEST well with an intensity greater than the Negative (-) Control well

Negative Result - No color reaction in the TEST well or color reaction less than or equal to the Negative (-) Control well

Equivocal Result - Reactions that cannot be determined as clearly positive or negative by the above criteria

INTERPRETATION OF RESULTS

A Positive Result indicates the specimen contains specific IgG/IgM antibodies at the test dilution. A positive reaction is comparable to an IgG titer of 1:32 or higher and/or an IgM titer of 1:16 or higher using a commercial test for antibodies at the test dilution. When correlated with clinical and laboratory findings, a positive result is indicative of an active or past infection with M. pneumoniae.

A Negative Result indicates detectable levels of specific IgG/IgM antibodies are not present in the specimen at the test dilution.

A specimen yielding an Equivocal Result should be retested after filtering the diluted sample through a 0.22 μm Millex GV Millipore syringe filter or equivalent. Use care to deliver only one drop of reagent at each step.

QUALITY CONTROL

The Positive (+) and Negative (-) Control wells contain inactivated M. pneumoniae antigens bound to the membrane which serve as reagent and procedural controls. Correct performance of controls, as shown below, indicates the test was performed correctly and all reagents and control sera are performing properly.

CONTROL WELL | EXPECTED RESULT
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Positive Control (+) | Development of a purple color upon completion of test
Negative Control (-) | No color or a faint purple color upon completion of test

If the Control wells (+ and –) fail to react as described, a procedural error or deterioration of test reagents may have occurred.

Failure of the Positive (+) Control well to develop color may indicate deterioration of reagents due to improper storage, use of reagents beyond stated expiration date, or improper reagent or sample addition (sequence, volume).

If aberrant quality control results are noted, patient results should not be reported.

LIMITATIONS

1. If a specimen collected soon after symptoms appear is negative for IgG/IgM antibodies and symptoms of M. pneumoniae infection persist, a second specimen should be collected two weeks later and retested. A positive result on the second specimen suggests an acute infection.10

2. Specimens refrigerated for longer than 5 days or previously frozen may result in high background staining and equivocal or false results, if not filtered after dilution.

3. Carefully follow recommended volumes for delivery of specimens, controls, and reagents to obtain accurate results. Delivery of reagents or specimens at angles other than described or the use of micropipettes other than those provided may yield altered volumes and cause aberrant results.

4. Indicated incubation times must be strictly adhered to.

EXPECTED VALUES

In untreated patients the IgM antibody appears first, 3-6 weeks after infection, and gradually wanes thereafter. IgG antibody is generally detectable later than IgM antibody and, in most cases, it becomes the prominent late antibody response.

This test is only part of the overall scheme for detection of antibodies to M. pneumoniae.11 Test results must be interpreted in the context of the illness. Serologic testing should not be used indiscriminately to diagnose M. pneumoniae or as the sole basis for the use of antibiotic therapy. Undirected testing can lead to erroneous conclusions and can subject patients to expensive, unnecessary, and potentially hazardous therapy.

PERFORMANCE CHARACTERISTICS

Mycoplasma Pneumoniae IgG/IgM Antibody Test was evaluated with 220 samples in three separate studies. Study 1 included 100 clinically asymptomatic individuals with no history of respiratory infections during the 3 weeks preceding the test. Study 2 tested Mycoplasma Pneumoniae IgG/IgM Antibody Test in parallel with a commercial IFA test for detection of IgG and IgM antibodies to M. pneumoniae in 98 samples, with a commercial microtiter ELISA test used to resolve discrepant results. Study 3 included 21 samples from patients with respiratory infections tested in parallel with Mycoplasma Pneumoniae IgG/IgM Antibody Test and a commercial IFA test for antibodies to M. pneumoniae.11

Study 1

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<th>Resolved</th>
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<td>IgG/IgM Test System Pos</td>
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<tr>
<td>Neg</td>
<td>0 95</td>
<td>Neg</td>
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<tr>
<td>Relative Specificity without discrepant analysis = 95%</td>
<td>Relative Specificity without discrepant analysis = 98.6%</td>
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Study 2 and 3

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<td>IgG/IgM Test System Pos</td>
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<tr>
<td>Neg</td>
<td>6 64</td>
<td>Neg</td>
<td>3 67</td>
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<tr>
<td>Relative Sensitivity without discrepant analysis = 87.8%</td>
<td>Relative Sensitivity without discrepant analysis = 90.1%</td>
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<td>Relative Specificity without discrepant analysis = 94.3%</td>
<td>Relative Specificity without discrepant analysis = 99%</td>
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Combined Resolved Data from Studies 1, 2, and 3:

- Relative Sensitivity = 94.7%
- Relative Specificity = 99.4%
- Positive Predictive Value = 98.2%
- Negative Predictive Value = 98.2%

The samples used in this study included fresh and banked sera from 1 healthcare university and 2 community hospitals located in different geographical regions. The assay results were not affected by hemolysis or low to moderate lipemia; however, grossly lipemic specimens should not be used.

BIBLIOGRAPHY