Mycoplasma Pneumonia
IgM IFA

Cat # 341010-M

PRINCIPLE OF THE ELISA ASSAY
The DAI indirect fluorescent antibody (IFA) test system is pre-standardized to detect the presence of circulating IgM antibodies to M. pneumoniae in human sera. M. pneumoniae antigenic substrate is affixed onto a multi-well microscope slide. Human serum to be tested is incubated with this substrate and antibody, if present, can be observed after staining with a fluorescein-labeled anti-human IgM conjugate. With proper illumination and filters, a fluorescence microscope will demonstrate characteristic positive, bright, apple-green fluorescence of the reaction.

SPECIMEN COLLECTION
1. It is recommended that specimen collection be carried out in accordance with NCCLS document M29: Protection of Laboratory Workers from Infectious Disease.
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, all blood derivatives should be considered potentially infectious.
3. Only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures should be used in this assay (13,14). No anticoagulants or preservatives should be added. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2°C and 8°C for no longer than 48 hours. If delay in testing is
anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles that may cause loss of antibody activity and give erroneous results.

EQUIPMENT AND MATERIALS
1. Small serological, Pasteur, capillary or automatic pipettes.
2. Small test tubes: 13 x 100, or comparable.
3. Test tube racks.
4. Staining dish: A large staining dish provides an ideal mechanism for washing slides between incubation steps.
5. Moist chamber.
6. Cover slips: 24 x 60mm, thickness No. 1.
7. Distilled water.
8. Properly equipped fluorescence microscope.

The following filter systems or their equivalent have been found to be satisfactory for routine use with transmitted or incident light darkfield assemblies:

<table>
<thead>
<tr>
<th>TRANSMITTED LIGHT</th>
<th>Light Source: Mercury vapor 200W or 50W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Filter</td>
<td>Barrier Filter</td>
</tr>
<tr>
<td>KP490</td>
<td>K510 or K530</td>
</tr>
<tr>
<td>BG12</td>
<td>K510 or K530</td>
</tr>
<tr>
<td>FITC</td>
<td>K520</td>
</tr>
<tr>
<td>Light Source: Tungsten – Halogen 100W</td>
<td></td>
</tr>
<tr>
<td>KP490</td>
<td>K510 or K530</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>INCIDENT LIGHT</th>
<th>Light Source: Mercury Vapor 200, 100, 50 W</th>
</tr>
</thead>
<tbody>
<tr>
<td>Excitation Filter</td>
<td>Dichroic Mirror</td>
</tr>
<tr>
<td>KP500</td>
<td>TK510</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
</tr>
<tr>
<td>Light Source: Tungsten – Halogen 50 and 100 W</td>
<td></td>
</tr>
<tr>
<td>KP500</td>
<td>TK510</td>
</tr>
<tr>
<td>FITC</td>
<td>TK510</td>
</tr>
</tbody>
</table>

MATERIALS REACTIVE REAGENTS
1. M. pneumoniae substrate slides.
2. Goat anti-human IgM labeled with fluorescein-isothiocyanate (FITC) containing counterstain. 1.5mL, lyophilized.
3. Human M. pneumoniae IgM positive control serum containing human sera producing 4+ apple-green staining of the substrate slide. 0.5mL, lyophilized.
4. Human M. pneumoniae IgM negative control serum. Consists of human sera with no detectable fluorescence as determined by the IFA technique. 0.5mL, lyophilized.

NON-REACTIVE MATERIALS
1. Phosphate-buffered-saline (PBS) sufficient to make 4 liters.
2. Buffered glycerol: 3mL.

NOTE: All sera, antisera, and buffered glycerol contain a preservative which may be toxic if ingested.

PREPARATION OF REAGENTS
1. Phosphate-buffered-saline (PBS): Empty contents of one buffer packet into one liter of distilled
water. Mix until all salts are thoroughly dissolved.

2. Human M. pneumoniae IgM positive control serum. Reconstitute with 0.5mL distilled water. Use as reconstituted. Do not dilute.

3. Human IgM negative control serum. Reconstitute with 0.5mL distilled water.

4. Anti-human IgM (FITC) labeled conjugate. Reconstitute with 1.5mL distilled water. Alternately, aliquot in 0.5mL amounts and store at -20ºC in small screw-capped tubes. Use as needed. Do not refreeze once aliquots have been thawed. Conjugate is stable once reconstituted for 90 days. Frozen aliquots are stable for 6 months.

STORAGE REQUIREMENTS

1. M. pneumoniae substrate slides: Store at -20ºC.

2. Anti-human IgM globulin labeled with FITC: Store at 2-8ºC. Stable for 90 days after reconstitution. Aliquots frozen at -20ºC are stable for six months.

3. Positive and negative human M. pneumoniae IgM control sera: Store at 2-8ºC. Stable for 90 days after reconstitution. Aliquots frozen at -20ºC are stable for six months.

4. Phosphate-buffered-saline (PBS): Store packets at room temperature. Once rehydrated, store at 2-8ºC. Rehydrated PBS is stable for 30 days.

5. Buffered glycerol: Store at 2-8ºC.

NOTE:

1. All kit components are stable until the expiration date printed on the label provided the recommended storage conditions are strictly followed. Do not use beyond the expiration date.

2. Do not freeze and thaw reagents or patient samples more than once. Repeated freezing and thawing destroys antibody activity. Do not store in self-defrosting freezers.

QUALITY CONTROL

1. Positive, negative, and buffer controls should be run with each assay.

2. It is recommended that the positive and negative controls be read prior to evaluating test results. This will assist in establishing the references required to interpret the test sample. If controls do not appear as described, test results are invalid.

3. The negative control is characterized by the absence of fluorescence in the slurry particulate matter.

4. The positive control sill exhibit a 3+ to 2+ apple-green fluorescent staining intensity of the slurry particulate matter.

5. The intensity of observed fluorescence may vary with the microscope and filter used.

6. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations, or accrediting organizations.

PROCEDURE – STEPWISE

1. Remove slides from freezer and allow them to warm to room temperature (20-25ºC). Tear open the protective envelope and remove slides containing M. pneumoniae. DO NOT APPLY PRESSURE TO FLAT SIDES OF PROTECTIVE ENVELOPE.

2. Prepare patient sera at a 1:8 dilution in PBS.

3. Identify each well with the appropriate patient sera and controls. Only fresh or properly refrigerated sera or plasma obtained by proper venipuncture procedures should be employed in this procedure.

4. Using suitable dispenser (capillary, Pasteur, or automatic pipette), dispense one drop, or approximately 0.020mL of patient and control sera in the appropriate wells.
5. Incubate slides in a moist chamber at 37°C for 60 minutes.
6. Take slides from the moist chamber and remove excess sera from the wells by gently rinsing slides with PBS prior to placing in wash chamber. Immediately wash slides with gentle agitation for two, five minute intervals with a change of PBS.
7. Take slides from PBS solution and remove excess buffer. Dry mask around wells with blotter provided, being careful not to blot wells, and add one drop of FITC labeled anti-human IgM conjugate to each well.
8. Incubate slides in a moist chamber for 30 minutes at 37°C.
9. Repeat step 6 and dry mask around wells.
10. Apply 3-5 drops of mounting media to each slide (between the wells), and coverslip. It is recommended that slides be examined on the same day as testing. Examine slides immediately with an appropriate fluorescence microscope assembly. Note: If delay in examining slides is anticipated, seal coverslip with clear nail polish and store in refrigerator. Read test results within 24 hours.

**CALCULATIONS/REPORTING RESULTS**

The presence of IgM antibodies in a single serum sample provides strong evidence of a current infection.

Examine the slides to ascertain the relative staining intensity of the M. pneumoniae aggregates. A positive result appears as an apple-green, fluorescent staining of particulate matter. The rhodamine counterstain suppresses non-specific background fluorescence and imparts a reddish-orange granular field to negative sera. Strict comparison with known positive and negative sera of the appropriate antibody class will help to provide consistent and reproducible results. A suggested algorithm for atypical pneumonias is available in the product insert.

**EXPECTED VALUES**

<table>
<thead>
<tr>
<th>RESULTS</th>
<th>SIGNIFICANCE</th>
</tr>
</thead>
<tbody>
<tr>
<td>No fluorescence (equal to or less than the Negative control)</td>
<td>No detectable antibody to pneumoniae by IFA test.</td>
</tr>
<tr>
<td>Fluorescence intensity of ≥ 1+ at 1:8 screening dilution, but specimen at a later date not more than 1:16</td>
<td>Equivocal Results. Retest another to evaluate the possibility of a seroconversion.</td>
</tr>
<tr>
<td>Fluorescence intensity of &gt; 1+ at 1:16 or higher.</td>
<td>Active or recent infection with M. pneumoniae.</td>
</tr>
</tbody>
</table>

**PROCEDURE NOTES**

1. For in vitro diagnostic use.
2. The preservative may be toxic if ingested.
3. Each component of Product Series 17000M has been manufactured compared to a reference standard. Therefore, components from different kit lots or individually purchased components of the DAI Mycoplasma IgM IFA test system may be interchanged. Follow kit procedures carefully.
4. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
5. To avoid dislodging the substrate during the wash procedure, use gentle agitation.

6. The human serum controls are POTENTIALLY BIOHAZARDOUS MATERIALS. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg, and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, these products should be handled at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual “Biosafety in Microbiological and Biomedical Laboratories”: current edition; and OSHA’s Standard for Bloodborne Pathogens (19).

**LIMITATIONS OF THE PROCEDURE**

1. The end-point titration is the highest dilution showing a 1+ intensity of the substrate reaction.
2. Lipemic patient samples interfere with the substrate and reagents. The trained technician will be able to differentiate this reaction from the specific one.
3. Proteolytic enzymes in the test samples may denature the substrate or reagents. If a sample appears contaminated with microorganisms, avoid using, and obtain another sample.
4. No single laboratory test is diagnostic by itself. The test result should be weighed against the patient’s history, physical exam, and clinical symptoms.
5. Rheumatoid factor (RF) may cause false positive reactions in IgM test procedures. Therefore, all positive IgM tests should be tested for rheumatoid factor. If rheumatoid factor is present, it must be removed by 56°C heat IgG-RF aggregation, processed through a prepacked disposable ion exchange mini-column, or Staphylococcus protein A absorption, followed by buffer elutions and the test rerun for M. pneumoniae IgM. Alternatively, all serum samples can be processed through the same type columns prior to testing for the IgM.
6. Specimens with high IgG antibody activity may produce false negative IgM antibody tests due to competition between the higher affinity IgG antibody and IgM antibodies. This potential problem may be eliminated by prior removal of the IgG and/or rheumatoid factor prior to testing (15), using one of the following:
   - Gel filtration (16)
   - Absorption with protein A (13), or protein G (17).
   - Ion-exchange Chromatography System
   - Precipitation of IgG with anti-human IgG serum(18)
   - The use of IgG Removal Reagent.

7. Occasionally a test specimen will exhibit excessive non-specific fluorescence over the total cell population. If the specimen shows a sufficiently strong positive reaction then it may be possible to interpret the specific fluorescence through the excessive background fluorescence. If the specimen cannot be interpreted at the 1:8 dilution, the result is equivocal. Note: It may be possible to detect a positive reaction by evaluating such a specimen through serial dilutions.

8. In some cases high concentrations of IgM in patients serum may produce a slight nonspecific staining. This staining is distinguished from the specific staining observed in the test through the use of specific positive and negative controls.

9. A negative result does not rule out current M. pneumoniae infection since the specimen may have been collected before demonstrable antibody is present or after the antibody has decreased below detectable levels. Consequently, demonstration of elevated M. pneumoniae IgG titers in conjunction with specific M. pneumoniae IgM, increases the specificity of serological diagnosis.

10. The endpoint reactions may vary due to the type of microscope employed, the light source, age of bulb, filter assembly, and filter thickness.
REFERENCES


<table>
<thead>
<tr>
<th>Date Adopted</th>
<th>Reference No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>2004-01-20</td>
<td>DA-Myco.p-IFA-2010</td>
</tr>
</tbody>
</table>