**Mycoplasma pneumonia IgG Crown-Titre IFA**

**Cat #341010-CTG**

**PRINCIPLE OF THE ASSAY**
The DAI indirect fluorescent antibody (IFA) test system is pre-standardized to detect the presence of circulating IgG 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 IgG 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° 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
MATERIALS REQUIRED BUT NOT PROVIDED:
1. Small serological, Pasteur, capillary or automatic pipettes.
2. Small test tubes: 13 x 100mm, 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>
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<tbody>
<tr>
<td>Excitation Filter</td>
<td>Barrier Filter</td>
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<tr>
<td>KP490</td>
<td>K510 or K530</td>
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<tr>
<td>BG12</td>
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<td>FITC</td>
<td>K520</td>
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<td>K510 or K530</td>
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<table>
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<tr>
<th>Incident Light</th>
<th>Light Source: Mercury Vapor 200, 100, 50 W</th>
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<tbody>
<tr>
<td>Excitation Filter</td>
<td>Dichroic Mirror</td>
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<tr>
<td>KP500</td>
<td>TK510</td>
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<tr>
<td>FITC</td>
<td>TK510</td>
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<tr>
<td>Light Source: Tungsten – Halogen 50 and 100 W</td>
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<tr>
<td>KP500</td>
<td>TK510</td>
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<tr>
<td>FITC</td>
<td>TK510</td>
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MATERIALS PROVIDED
Reactive Reagents
1. \( M. pneumoniae \) substrate slides
2. Goat anti-human IgG labeled with fluorescein isothiocyanate (FITC), containing counterstain (lyophilized), 1.5mL
3. Human \( M. pneumoniae \) positive control serum containing human sera producing 4+ apple-green staining of the substrate slide (lyophilized), 0.5mL
4. Human \( M. pneumoniae \) negative control serum containing normal human sera with no detectable fluorescence as determined by the IFA technique (lyophilized), 0.5mL

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

NOTE: All reactive reagents, as well as buffered glycerol, contain a preservative which may be toxic if ingested. (Thimerosal, mercury derivative 1:10,000.)
STORAGE CONDITIONS
1. *M. pneumoniae* substrate slides: -20°C.
2. Anti-human IgG labeled with FITC: 2-8°C. Stable for 90 days after reconstitution. Aliquots frozen at -20°C are stable for six months.
3. Positive and negative human *M. pneumoniae* control sera: 2-8°C. Stable for six months.
4. Phosphate-buffered-saline (PBS): Store packets at room temperature. Once rehydrated store at 2-8°C. Rehydrated PBS is stable for 30 days.
5. Buffered glycerol: Store at 2-8°C.

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. A positive control, negative control, and buffer control 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 will exhibit a 4+ to 3+ apple-green fluorescent staining intensity of the slurry particulate matter.
5. The intensity of the 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
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* positive control serum. Reconstitute with 0.5ml distilled water. Use as reconstituted. Do not dilute.
3. Human negative control serum: Reconstitute with 0.5ml distilled water.
4. Anti-human IgG (FITC) labeled conjugate: Reconstitute with 1.5ml of distilled water. Alternately, aliquot in 0.5ml amounts and store at -20°C 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.

TEST PROCEDURE
1. Remove slides from freezer, and allow them to reach room temperature (20-25°C). 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 1:64 dilution in PBS.
3. Identify each well with the appropriate patient sera and controls. Only fresh or properly refrigerated serum 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 room temperature (20-25°C) for 30 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 without agitation for two, 5
minute intervals with a change of PBS. **Note:** Stirring or agitation may dislodge colonies.

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 immunoglobulin conjugate to each well.

8. Incubate slides in a moist chamber for 30 minutes at ambient temperature (20-25°C).

9. Repeat step 6 and dry mask, using blotter provided.

10. Apply 3-5 drops of mounting media to each slide (between the wells) and gently apply coverslip. Do not apply pressure to coverslip. It is recommended that slides be examined on the same day of 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 INTERPRETATION:**
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 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 shown in Table 5.

**PROCEDURE NOTES**
1. For *in vivo* diagnostic use.
2. The preservative may be toxic if ingested.
3. Do not apply pressure to slide envelope, this may damage the substrate.
4. The components of this kit are matched for optimum sensitivity and reproducibility. Reagents from other kits or sources should not be interchanged. Follow kit procedures carefully.
5. Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
6. To avoid dislodging the substrate during the wash procedure, use gentle agitation.
7. 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 (15).
8. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
9. Avoid microbial contamination of reagents. Incorrect results may occur.
10. Cross-contamination of reagents and/or samples could cause false results.

**LIMITATIONS OF THE PROCEDURE**
1. To confirm acute infection, paired samples are required. A first specimen should be obtained once manifestations occur (acute). A second specimen should be obtained approximately two weeks later (convalescent). A four-fold increase in antibody titer is considered diagnostic for a current infection if tested simultaneously.
2. The endpoint titration is the highest dilution showing a 1+ intensity of the substrate reaction.
3. Lipemic patient samples interfere with the substrate and reagents. The trained technician will be able to differentiate this reaction from the specific one.
4. Proteolytic enzymes in the test samples may denature the substrate or reagents. If a sample appears contaminated with microorganisms, avoid using and try to obtain another, more suitable sample.
5. No single laboratory test is diagnostic in itself. The test result should be weighed against the patient’s history, physical exam, and clinical symptoms.
6. 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>
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<tr>
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<tr>
<td>2004-01-20</td>
<td>DA-Mycoplasma Pneumonia IgG- Crown Titre-IFA-2010</td>
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