



DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite E/F, Calabasas, CA 91302

Tel: (818) 591-3030 Fax: (818) 591-8383

onestep@rapidtest.com

technicalsupport@rapidtest.com

www.rapidtest.com



See external label



2°C-8°C



Σ= tests



cat. #341010-G

Mycoplasma IgG Test System

Catalog No. 341010-G

PLEASE READ THIS MATERIAL BEFORE USING THE KIT

INTENDED USE

The Diagnostic Automation. Mycoplasma IgG Test System is designed for the qualitative and semi-quantitative detection of *Mycoplasma pneumoniae* IgG antibodies in human serum and is for *in vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Mycoplasma pneumoniae is the most common cause of pneumonia and febrile upper-respiratory tract infections in the general population (except for influenza A) (1-5). Other nonrespiratory complications may also develop with this disease in virtually any organ system, with insult ranging from mild to life-threatening (6,8).

Mycoplasma pneumoniae, a prokaryote, is the smallest (10 x 200nm), and simplest self-replicating microorganism known, and more closely resembles a bacterium rather than a virus. However, because it lacks a cell wall, a resistance to cell-wall-active antibiotics is obvious (i.e., penicillin, cephalosporins) (1). This concern for diagnostic, or at least therapeutic accuracy in the early management of community-acquired infections is particularly critical in very young or elderly patients where very little temporal margin of error exists.

Until recently, the routine laboratory diagnosis of this infection has been limited to insensitive and/or non-specific assays (i.e., cold agglutinins, complement-fixation, culture isolation). Species-specific antibodies to surface antigens are now known to exist. They are protective, and are readily detected by immunofluorescence; even in the early stages of the disease (9). The diagnosis therefore, is best achieved serologically (10), and with immunofluorescence, the test can separate current immune response to infections from that of previous exposure (11).

PRINCIPLE OF THE ASSAY

The Diagnostic Automation. 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.

KIT COMPONENTS

Reactive Reagents:

- *M. pneumoniae* substrate slides (Product #:17002).
- Goat anti-human IgG labeled with fluorescein isothiocyanate (FITC), containing rhodamine counterstain (lyophilized), 1.5mL

(Product #:17003).

- Human *M. pneumoniae* positive control serum containing human sera producing 4+ apple-green staining of the substrate slide (lyophilized), 0.5mL (Product #:17004).
- Human *M. pneumoniae* negative control serum containing normal human sera with no detectable fluorescence as determined by the IFA technique (lyophilized), 0.5mL (Product #:17005).

Non-Reactive Materials:

- Phosphate-buffered-saline (PBS) - sufficient to make 4 liters (Product #:0008).
- Buffered glycerol, 3mL (Product #:0009).

NOTE: All reactive reagents, as well as buffered glycerol, contain a preservative which may be toxic if ingested. (Thimerosal, mercury derivative 1:10,000.)

PRECAUTIONS

- For *in vitro* diagnostic use.
- The preservative may be toxic if ingested.
- Do not apply pressure to slide envelope, this may damage the substrate.
- 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.
- Reconstitute reagents gently but thoroughly. Reagents should be free of particulate matter. If reagents become cloudy, bacterial contamination should be suspected.
- To avoid dislodging the substrate during the wash procedure, use **gentle** agitation.
- Each donor unit used in the preparation of the controls was found to be negative when tested by an FDA approved method for the presence of HBsAg, and for antibodies to HIV-1, HIV-2, and HCV.

WARNING - POTENTIAL BIOHAZARDOUS MATERIAL

Because no test method can offer complete assurance that human immunodeficiency virus, hepatitis B virus, or other infectious agents are absent, these specimens/reagents, as well as patient samples, 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 Microbiology and Biomedical Laboratories", 1984, p.12-16, 3rd edition- 1993, and OSHA Standard for Bloodborne Pathogens (15).

- Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
- Avoid microbial contamination of reagents. Incorrect results may occur.
- Cross-contamination of reagents and/or samples could cause false results.

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ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

- Small serological, Pasteur, capillary or automatic pipettes.
- Small test tubes: 13 x 100mm, or comparable.
- Test tube racks.
- Staining dish: A large staining dish provides an ideal mechanism for washing slides between incubation steps.
- Moist chamber

SPECIMEN COLLECTION

Only freshly drawn and properly stored blood 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.

Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored at 2-10° 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 which may cause loss of antibody activity and give erroneous results.

STORAGE CONDITIONS

- *M. pneumoniae* substrate slides: -20°C.
- 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.
- Positive and negative human *M. pneumoniae* control sera: 2-8°C. Stable for six months.
- Phosphate-buffered-saline (PBS): Store packets at room temperature. Once rehydrated store at 2-8°C. Rehydrated PBS is stable for 30 days.
- 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.

PROCEDURE**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.

- Cover slips: 24 x 60mm, thickness No. 1.
- Distilled water.
- Properly equipped fluorescence microscope.

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.

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.

INTERPRETATION OF RESULTS

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 shown in Table 5.

LIMITATION OF THE ASSAY

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.

employed, the light source, age of bulb, filter assembly and filter thickness.

6. The endpoint reactions may vary due to the type of microscope

EXPECTED RESULTS/SIGNIFICANCE

NOTE: The negative control provided with this test system should be used as a guide in determining fluorescence intensity. Reactions with a fluorescence intensity greater than that of the negative control are to be reported in the following manner:

Table 2

Calculation of Initial Sensitivity and Initial Specificity

Initial Sensitivity = $8/9 = 89\%$, with 1 discrepant specimen (see Table 3).

Initial Specificity = $118/124 = 95\%$, with 6 discrepant specimens (see Table 3)

Table 4

Calculation of Relative Sensitivity and Relative Specificity, and Percent Agreement After Resolution of discrepant Specimens:

RESULTS

SIGNIFICANCE

No fluorescence (equal to or less than the negative control) No detectable antibody to pneumoniae by IFA test.

Fluorescence intensity of $\geq 1+$ at 1:64 screening dilution, but not more than 1:128 Equivocal Results. Retest another specimen at a later date to evaluate the possibility of a seroconversion.

Fluorescence intensity of $> 1+$ at 1:128 or higher Active or past infection with *M. pneumoniae*.

It is recommended that in the event of borderline interpretations further testing be performed to evaluate the possibility of a later seroconversion.

PERFORMANCE CHARACTERISTICS

In a clinical study conducted by Diagnostic Automation., 143 patient specimens were tested by complement fixation (CF), the Diagnostic Automation. Mycoplasma IgG IFA Test System, and the Diagnostic Automation. Mycoplasma IgM Test System. The results of the study have been depicted in Tables 1 through 4 below:

Table 4

Calculation of Relative Sensitivity and Relative Specificity, and Percent Agreement after resolution of discrepant specimens.

Relative Sensitivity	=	12/12	=	100.0%
Relative Specificity	=	118/121	=	97.5%
Percent Agreement	=	130/133	=	97.7%

Table 1

Results of the Diagnostic Automation. Mycoplasma IFA test systems compared to CF
Complement fixation Result^a

		Positive	Negative	Borderline ^c
DAIIFA Results ^b	Positive	8	6	5
	Negative	1	118	2
	Borderline ^c	0	3	0

^a CF is a polyvalent test and therefore will not distinguish between specific IgG and/or IgM antibodies.

^b The Diagnostic Automation. IFA results were determined using the following interpretations:

Negative: IgG titer of $\leq 1:32$ and IgM titer of $\leq 1:4$.

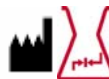
Positive: IgG titer of $\geq 1:128$ and/or IgM titer of $\geq 1:16$.

Borderline: IgG titer of 1:64 and/or IgM titer of 1:8.

^c Borderline specimens were excluded from all calculations

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