IMMUNOFLOW™

MYCOPLASMA PNEUMONIAE IGM TEST

REF 7005 Σ 25 CE

For In Vitro Diagnostic Use

INTENDED USE

ImmunoFLOW *Mycoplasma pneumoniae* IgM Test detects human IgM to the complement-fixing antigen and is intended as an aid in the diagnosis of infection.

SUMMARY

The order Mycoplasmatales includes approximately 70 species, most of which are not found in humans. The genus *Mycoplasma* contains two species commonly found in man, *M. pneumoniae* and *M. genitalium*. These two species share lipid antigen specificities (complement-fixing antigen), and are therefore antigenically related. Two other human pathogens, *M. hominis* and *Ureaplasma urealyticum* are not serologically related and therefore not cross-reactive to this complement-fixing antigen.

M. pneumoniae is the only known *Mycoplasma* species that is a primary pathogen in man. Clinical manifestations can range from asymptomatic respiratory infections to severe pneumonia. *M. pneumoniae* accounts for 15 to 20% of total pneumonia. Other symptoms associated with *M. pneumoniae* infection include abnormalities of the central nervous system (meningitis, encephalitis), cardiac involvement (myocarditis, pericarditis), hemolytic anemia, arthritis, G.I. inflammations, and mucocutaneous reactions. *M. pneumoniae* is identified as a common infectious cause of Stevens-Johnson Syndrome, a well-defined systemic disease that can develop into a life-threatening illness in children.

The *M. pneumoniae* organism is sensitive to erythromycin and tetracyclines; however, it is resistant to drugs more routinely given in the treatment of acute pneumonia. Thus, a rapid and reliable diagnosis of *M. pneumoniae* infection is essential to proper patient management. *M. pneumoniae* culture is difficult, slow and relatively insensitive. Nucleic acid detection methods can be sensitive but require localized specimen collection (for example, lower lung lavage). Serology is the primary diagnostic tool. Serological methods are complement fixation (CF), indirect immunofluorescence assays (IFA), immune adherence hemagglutination assay (IAHA) and enzyme immunosorbent assays (EIA).

ASSAY PRINCIPLE

ImmunoFLOW is an immunoassay consisting of a cassette and three reagents. The cassette contains a paper matrix (for example, nitrocellulose) and an absorbent material. The paper matrix was manufactured with three “dots”, each contain an antigen (for example, positive control, analyte 1, analyte 2). A body fluid (for example, serum) is applied to the triangular opening an allowed to flow through the paper matrix into the absorbent. To assure assay specificity, a wash reagent is applied and flows into the absorbent material. Finally gold particles attached to an immunological reagent (e.g., anti-immunoglobulin) is applied and absorbed.

The “dot” applied to the paper matrix contains antigen. Specific antibody in body fluid will bind to the antigen. If specific antibody binding occurs, immunologically active gold particles will bind and cause a red/pink color formation.

REAGENTS

Cassette: *M. pneumoniae*, strain FH (ATCC #15531), complement-fixing antigen and reagent control

Wash 2: Buffered solution with <0.1% sodium azide

Diluent: Buffered saline with <0.1% sodium azide

Color M: Colloidal gold conjugated to goat anti-human IgM in buffered saline with <0.1% sodium azide

WARNINGS AND PRECAUTIONS

Some reagents contain sodium azide (NaN3) that may react with lead and copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide buildup.

Human sera used in the preparation of this product were tested and found non-reactive for hepatitis B surface antigen and for antibodies to HIV-1, HIV-2, HTLV-1, and hepatitis C virus. Because no test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease.

STORAGE

Store at 2-8°C. Bring reagents to room temperature (15-30°C) before use. Avoid contamination of reagents. Once the foil pouch containing one cassette is opened, it should be used within 12 hours. Other kit components are to be used prior to the expiration date.

COLLECTION AND HANDLING

ImmunoFLOW Test is performed on serum. Store samples at room temperature for no longer than eight hours. If the assay will not be completed within eight hours, refrigerate the sample at 2-8°C. IgM activity may be affected by freezing the specimen.

PROCEDURE

MATERIALS PROVIDED

Cassette	Color M
Wash 2	Diluent

MATERIALS REQUIRED BUT NOT PROVIDED

Collection apparatus	Timer
Pipette	Test tubes
Control Reagents	Camera (optional)

SET-UP

1. Remove cassette(s) 1 per test.
2. Remove wash reagent (Wash 2), color developer (Color M).
3. Prepare a 1:2 (50:50) dilution of the patient specimen or control using specimen diluent (Diluent). (For example, add 100 µL patient sample into 100 µL Diluent).

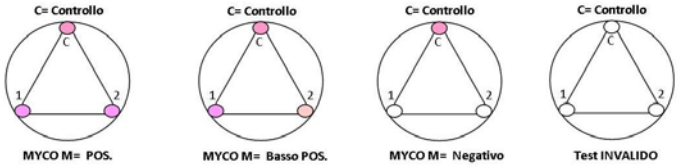
ASSAY PROCEDURE

1. Add **one hundred (100) micro liters (µL)** of Wash 2 to the cassette. Allow all liquid to flow through device.
2. Add **100 µL** of 1:2 **diluted** patient or control specimen. Allow all liquid to flow through the device. (Note: If sample takes longer than one (1) minute to flow through cassette, do not continue. Attempt run once more. If sample continues to not flow, this sample is not acceptable for procedure. If this commonly occurs, report problem to GenBio. Typically this is caused by highly lipemic sera, but may occur without apparent cause.)
3. Add **100 µL** of Wash 2 to the cassette. Allow all liquid to flow through device.
4. Add **100 µL** of color developer (Color M) to the cassette. Allow all liquid to flow through device.
5. Add **100 µL** of Wash 2 to the cassette. Allow all liquid to flow through device.
6. Read the results within **two (2) minutes**. (A permanent record may be made using a digital camera.)

INTERPRETATION

Reactive	A red dot is visible
Not Reactive	No dot is visible

It is recommended that users initially testing a series of presumptive negative specimens. If needed, GenBio can provide a positive specimen (Part Number 800-1251). Please contact GenBio's Technical Service.



CLINICAL INTERPRETATION

Positive	All dots reactive
Low Positive	Control and dot 1 reactive
Negative	Only control dot reactive
Do Not Interpret	No dots reactive

Use caution when interpreting low dot intensity. If intensity is low, Dot 1 must report stronger color intensity than Dot 2. For best clinical correlation, if not certain, interpret as negative.

QUALITY CONTROL

The assay is performed at room temperature (18-27°C).

Testing should be according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations. Unless otherwise required, it is recommended that control sera be tested upon receipt of a kit.

Each cassette includes a reagent positive control. The background area around the dot is the reagent negative control. The reagent positive control dot must be reactive and the background must be white to pale pink. If the reagent control dot is not reactive or the background is reactive, do not interpret the assay strip.

It is recommended an initial assay validation using positive and negative control serum be done before introducing the test into the facility. This is done by testing both negative and positive specimens. GenBio can provide borderline and low positive validation samples to help validate and assure user interpretation training. Please contact. It is also recommended that the facility these negative and positive samples upon receipt of a new shipment. [Phase 1 samples perform this validation step.]

LIMITATIONS

The values obtained in the assay are intended to be an aid to diagnosis only. Clinical interpretation also requires consideration of the patient's history, physical findings and other diagnostic procedures.

M. pneumoniae complement-fixing antigen may cross-react with other *Mycoplasma* IgM specific antibodies.

Results obtained from immunocompromised individuals should be interpreted with caution.

The performance characteristics have not been established for any matrices other than serum.

There is a possibility of assay cross-reactivity with specimens containing rheumatoid factor.

The prevalence of the analyte will affect the assay's predictive value.

EXPECTED RESULTS

Specific IgM may be present in specimens collected from healthy ("presumptive negative") subjects. Low amounts of specific IgM is present in some healthy subjects. Eighty-three specimens collected in a U.S. asymptomatic adult population were tested using an enzyme-immunoassay (EIA) (ImmunoWELL M. pneumoniae IgM Test) and ImmunoFLOW. One specimen reported EIA positive (1050 units/mL) and two specimens reported EIA low positive (807 and 761 units/mL) and all three reported ImmunoFLOW low positive. The remaining specimens reported EIA negative. One presumptive negative specimen (481 units/mL) was ImmunoFLOW low positive yielding relative specificity of 99% (80/81).

PERFORMANCE

ImmunoWELL Mycoplasma IgM Test uses the same complement-fixing antigen and was validated using case-defined acute and convalescent specimens. Using ImmunoFLOW as reference, specimens were supplied to four sites. Sites A, B and C are located in the European Union. Site D is GenBio. Six replicates of each specimen listed in Table 2 were tested. The same specimens were provided to all sites.

Sensitivity, relative to EIA activity, is 100% (96/96) (Table 1: Performance Related to IgM Activity).

Reproducibility is 100% (144/144) for specimens reporting values significantly above or below the clinical cutoff region. Two borderline specimens (853 and 644 units/mL) respectively reported 77% and 65% reactivity. Interpretation of borderline reactivity depends on the technician's interpretation. Data presented in Table 1 report results of eight technicians. Interpretation of the borderline specimens ranges for 0-100% reactivity.

Table 1: Performance Related to IgM Activity

Units/mL IgM	Interpretation	Percent Positive or Low Positive
1477	Positive	100% (48/48)
1399	Positive	100% (48/48)
853	Low Positive	77% (37/48)
644	Negative	65% (31/48)
37	Negative	0% (0/48)

SIMBOLS

	Use by Date	IVD	In vitro diagnostic medical device
LOT	Batch Code	EC REP	Authorized Representative in the Europern Community
	Caution, consult Accompanying documents		Limit of Temperature
	Manufacturer	REF	Catalogue Number
	Contains sufficient for <n> tests	CONT	Contents
	Do not reuse	CE	Conformite Europeenne

Manufacturer

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