# *ImmunoWELL™*

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## MYCOPLASMA PNEUMONIAE ANTIBODY (IgG) TEST

**Product No. 3120**

IVD **For *In Vitro* Diagnostic Use**

SEE CALIBRATION VALUES TABLE 1, PAGE 3

## INTENDED USE

ImmunoWELL Mycoplasma Pneumoniae Antibody (IgG) Test is a semi–quantitative or qualitative determination of IgG antibodies in human serum to Mycoplasma pneumoniae for the determination of immunological experience. The test may be used to evaluate paired sera for the presence of seroconversion and a significant increase in specific IgG and in the diagnosis of Mycoplasma pneumoniae infection in the adult population.

## SUMMARY AND EXPLANATION

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, and are therefore antigenically related. Two other human pathogens, M. hominis and Ureaplasma urealyticum are not serologically related to these.

Mycoplasma 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 (1). M. pneumoniae accounts for 15 to 20% of total pneumonia (2) (3). 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 (4). Mycoplasma 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 (5).

The Mycoplasma 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 (6). Culturing of M. pneumoniae is too difficult and slow for clinical diagnostic utility. Serology provides the primary diagnostic tool with current methods including complement fixation (CF), indirect immunofluorescence assays (IFA), immune adherence hemagglutination assay (IAHA) and enzyme immunosorbent assays (EIA).

## ASSAY PRINCIPLE

The ImmunoWELL Test utilizes an EIA microtiter plate technique for the detection of antibodies. Serum is added to antigen coated microtiter wells and allowed to react. After removal of unbound antibodies, horseradish peroxidase-conjugated antihuman IgG antibodies are allowed to react with bound antibodies. The bound peroxidase reacts with 2,2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS®), the chromogenic substrate, developing a color. Finally, the substrate reaction is stopped and the optical density is read with a spectrophotometric microwell reader.

## REAGENTS

**Reaction Wells** coated with Mycoplasma pneumoniae, strain FH (ATCC #15531). The antigen is purified by chloroform and methanol extraction.
**Specimen Diluent** consisting of 0.01 M phosphate buffered saline (PBS, pH 6.2-7.6) and carrier protein containing <0.1% $NaN_3$
**Calibrator** consisting of human anti–M. pneumoniae prediluted 1:100 in Specimen Diluent
**Positive Control** consisting of human anti–M pneumoniae serum containing <0.1% $NaN_3$
**Negative Control** consisting of a nonreactive serum substitute containing <0.1% $NaN_3$
**Wash Buffer** Concentrate consisting of a 20X concentrate of 0.01 M PBS (pH 6.2-7.6) and 0.05% Tween
**Conjugate** consisting of peroxidase-conjugated goat antihuman IgG in PBS (pH 6.2-7.6) and carrier protein containing preservatives
**Substrate Buffer** consisting of 0.1 M sodium citrate (pH 4.4-4.6) and 0.01% hydrogen peroxide
**Substrate Concentrate** 2.19% 2-2'-azino-di-[3-ethylbenzthiazoline sulfonate] (ABTS) in 0.1 M sodium citrate (pH 4.4-4.6)
**Stop Solution** 0.25 M Oxalic Acid

## WARNINGS AND PRECAUTIONS

**For *In Vitro* Diagnostic Use:** ImmunoWELL reagents have been optimized for use as a system. Do not substitute other manufacturers' reagents or other ImmunoWELL Test reagents. Dilution or adulteration of these reagents may also affect the performance of the test. Do not use any kits beyond the stated expiration date. Close adherence to the test procedure will

assure optimal performance. Do not shorten or lengthen stated incubation times since this may result in poor assay performance.
Sodium azide may react with lead and copper plumbing to form highly explosive metal azides. It may be harmful if enough is ingested (more than supplied in kit). On disposal of liquids, flush with a large volume of water to prevent azide build-up (7). This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.
0.25M Oxalic acid [~3% C2H2O4], CAS# 144-62-7, EC No 205-634-3 may be harmful if swallowed so handle according to GLP. This dilution is not subject to GHS, US HCS and EU Regulation 2008/1272/EC labeling requirements.
The safety data sheet (SDS) is available at support.genbio.com or upon request.

**Human source material**. Material used in the preparation of this product has been tested and found non-reactive for hepatitis B surface antigen (HBsAg), antibodies to hepatitis C virus (HCV), and antibodies to human immunodeficiency virus (HIV-1 and HIV-2). Because no known test method can offer complete assurance that infectious agents are absent, handle reagents and patient samples as if capable of transmitting infectious disease (8). Follow recommended Universal Precautions for bloodborne pathogens as defined by OSHA (9), Biosafety Level 2 guidelines from the current CDC/NIH Biosafety in Microbiological and Biomedical Laboratories (10), WHO Laboratory Biosafety Manual (11), and/or local, regional and national regulations.

## RECONSTITUTION AND STORAGE

**Kit** is stored at 2-8°C. Assuming good laboratory practices are used, opened reagents remain stable as indicated by the expiration date.
**Reaction wells** are removed from the foil pouch and unused wells are resealed in the pouch using the integral zip-lock.
**Wash Buffer** (pH 6.2-7.6) is prepared by adding the contents of the Wash Buffer Concentrate (20X) bottle into 1 liter of distilled/deionized water. After reconstitution, the 1X solution is stored at 2-8°C. Discard when visibly turbid.
**NOTE: IN SOME INSTANCES THE WASH BUFFER CONCENTRATE (20X) MAY DEVELOP CRYSTALS UPON STORAGE AT 2-8°C. IT IS IMPORTANT THAT THESE CRYSTALS ARE COMPLETELY REDISSOLVED BEFORE DILUTION OF THE CONCENTRATE. THIS CAN BE ACCOMPLISHED BY WARMING THE CONCENTRATE TO 37°C IN A WATER BATH WITH OCCASIONAL MIXING.**
**Color Developer** is prepared by adding one (1) drop of Substrate Concentrate to 1mL of Substrate Buffer. One mL of Color Developer is sufficient for one eight-well strip. **Use within one hour.**

## SPECIMEN COLLECTION AND HANDLING

ImmunoWELL Test is performed on serum. The test requires 10 µL of serum. Lipemic, hemolyzed and icteric serum have not been shown to be acceptable specimens. Serum is collected according to standard practices and may be stored at 2–8°C for up to five days. Serum may be frozen below -20°C for extended periods.
Two sera, one collected during the acute disease phase and the other collected at least one week later (convalescent), are used.

# PROCEDURE

## MATERIALS PROVIDED

| | |
|---|---|
| Microtiter Wells in carrier | Specimen Diluent |
| Positive Control | Calibrator |
| Negative Control | Conjugate |
| Wash Buffer Concentrate (20X) | Substrate Concentrate |
| Substrate Buffer | Stop Solution |

## MATERIALS REQUIRED BUT NOT PROVIDED

| | |
|---|---|
| Distilled or deionized water | Test tubes |
| Microwell washer | Pipets |
| Microwell spectrophotometer (405 nm) | |

## PERFORMANCE CONSIDERATIONS

Reproducibility in the assay is largely dependent upon the consistency with which the microwells are washed. Carefully follow the recommended washing sequence as outlined in the assay procedure.
**Positive and Negative Control Sera (undiluted)** - These control sera are used to assure test performance.
**Calibrator (prediluted 1:100**) is used to standardize between run values.
**Substrate Blank** - All reagents, except serum, are added to the substrate blank well. This blank well is intended to baseline (zero) the microwell spectrophotometer.

## ASSAY PROCEDURE

1. Allow all components including diluted Wash Buffer to warm to room temperature (22-27°C).
2. Determine the total number of specimens to be run. Include one blank and duplicates of calibrator and controls in each run.
3. For each control and specimen, pipet 10 µL serum into a clean tube containing 1 mL Specimen Diluent and mix (1:100 dilution).

*CAUTION: Calibrator is prediluted. Do not dilute further.*

4. Determine the total number of wells to be run including blank, calibrators, controls and specimens. Well strips can be broken to the exact number needed to conserve reagent wells. Strips need to be completed with used wells to facilitate washing procedures.
5. Add 100 µL of Specimen Diluent into the first well as a substrate blank.
6. Pipet 100 µL of the prediluted calibrator and the diluted controls and specimens into each assigned well.
7. Incubate at room temperature (22-27°C) for 60±2 minutes.
8. Aspirate the samples out of the wells.
9. Wash the wells three times by completely filling the wells with Wash Buffer (see Reconstitution and Storage) and aspirating the wells completely after washes.
10. Pipet 100 µL Conjugate into all wells.
11. Incubate the wells at room temperature (22-27°C) for 30±2 minutes.
12. Aspirate the conjugate out of the wells.
13. Wash the wells three times as described in step 9.
14. Prepare fresh Color Developer (see Reconstitution and Storage).
15. Pipet 100 µL of Color Developer into each well.
16. Incubate at room temperature (22-27°C) for 30±2 minutes.
17. Add 100 µL of Stop Solution to each well.
18. Inspect the outside bottom surface of the microwells for the presence of condensation, dried buffer salts or wash solution which might interfere with the spectrophotometric reading. Carefully clean the well bottoms with a soft tissue.
19. Using the substrate blank to zero the spectrophotometer, read the optical density of each well at 405 nm within 30 minutes of completion of step 17.

IT IS RECOMMENDED THAT DUAL WAVELENGTH SPECTROPHOTOMETERS USE ONLY ONE WAVELENGTH, 405 NM.

## QUALITY CONTROL

GenBio provides positive and negative controls with defined ranges (see **Table 1**). Interpretations should not be made unless the control results fall within these limits. In addition, the laboratory should act in accordance with laboratory accreditation requirements and/or individual laboratory monitoring programs.

## INTERPRETATION

In order to eliminate the effects of washing variation, instrument variability, etc., specimen values are normalized according to the following calculation:

$V_S = A_S \times V_{MC}/A_{MC}$

Where:

$V_S$ = Value of the specimen (U/mL)

$A_S$ = Absorbance of the specimen

$V_{MC}$ = Assigned Value of the Mid Calibrator (U/mL)

**Table 1: Calibration Values**

| | Values | Units |
|---|---|---|
| **Calibrator Assigned Value** | 1695 | Units/mL |
| **Calibrator Low Limit** | 0.42 | Absorbance |
| | | |
| **Positive Control Expected Value** | 200-1800 | Units/mL |
| **Negative Control Expected Value** | <200 | Units/mL |

Results should not be interpreted if calibrator absorbance is below the low limit.

## SINGLE SPECIMEN METHOD

The activity is interpreted and reported as follows:

| | Interpretation |
|---|---|
| <100 | Result Negative, No detectable IgG antibody - Suggests no prior immunological exposure to Mycoplasma pneumoniae. Result does not rule out recent exposure and collection of test sample prior to development of IgG. Culture is recommended for determining current infection. |
| 100-320 | Equivocal - Immunological exposure cannot be assessed. Samples that remain equivocal after repeat testing should be retested by an alternate method, e.g., immunofluorescence assay (IFA). If results remain equivocal upon further testing, an additional sample should be taken. |
| >320 | Positive Detectable IgG Mycoplasma antibody - Indicates presence of detectable IgG antibody. Suggests immunological exposure to Mycoplasma pneumoniae. |

## SEROCONVERSION METHOD (ONE OR BOTH SAMPLES <200 UNITS)

Values below 200 units are inherently imprecise and should not be used to calculate ratios. If either IgG value is below 200 units, the seroconversion method should be used. Otherwise, the ratio calculation method should be used. A significant change (seroconversion) is indicated if one sample is above 320 units and the other is below 200 units. (See Table 2)

**Table 2: Seroconversion Interpretation**

| Acute | Convalescent | Indication |
|---|---|---|
| ≤200 | >320 | Significant M. pneumoniae antibody rise indicating current or recent infection |
| ≤200 | ≤320 | No significant M. pneumoniae antibody change detected |
| >320 | ≤200 | Significant M. pneumoniae antibody fall indicating recent past infection |

## RATIO METHOD (NEITHER SAMPLE <200 UNITS)

A ratio is calculated by dividing the convalescent sample result by the acute sample result. Either absorbance values or activities (U/mL) may be used to calculate the ratio. Interpretations are made as described in Table 3. If both values are above 1500 units, no interpretation is possible since the assay is not reliably linear beyond 1500 units.

**Table 3: Ratio Method Interpretation**

| Ratio | Indication |
|---|---|
| ≥2.0 | Significant M. pneumoniae antibody rise indicating current or recent infection. |
| ≤0.5 | Significant M. pneumoniae antibody change detected |
| >0.5 and <2.0 | No significant M. pneumoniae antibody change detected |

Examples of both methods are shown in Table 4.

**Table 4: Examples**

| Acute | Convalescent | Indication |
|---|---|---|
| 260 | 977 | (Ratio – 3.8) Significant M. pneumoniae antibody rise indicating current or recent infection |
| 260 | 384 | (Ratio = 1.5) No significant M. pneumoniae antibody change |
| 1893 | 256 | (Ratio = 0.1) Significant M. pneumoniae antibody fall indicating past infection |
| 456 | 134 | (Seroconversion) Significant M. pneumoniae antibody fall indicating recent past infection |

It is unknown whether IgG antibody cross-reactivity may occur with other organisms that cause similar symptomatology. It is recommended that assays to detect other agents or antibodies to other agents always be performed in conjunction with this assay.

The M. pneumoniae antibody levels obtained from the assay are an aid to diagnosis only. In most cases, a positive antibody result will provide laboratory support of M. pneumoniae infection. However, specific IgM may persist for several months after initial infection or be absent during early infection or reinfection. Each physician must interpret these results in light of the patient's history, physical findings, and other diagnostic procedures.

## LIMITATIONS

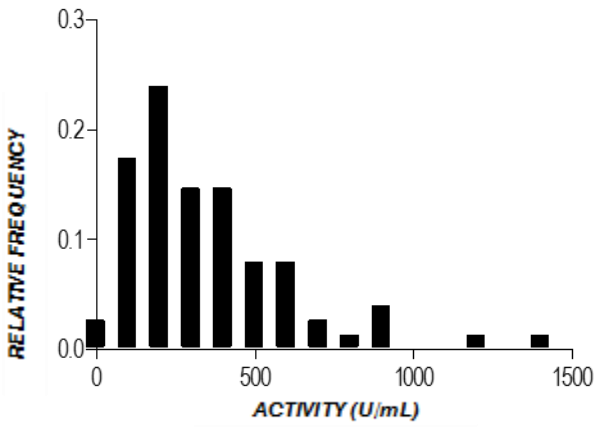
The state of present-day technology does not provide a recommended reference standard. Because of the current inconsistencies in various test methodologies, physicians and laboratories must rely on a combination of test methods, results

and clinical symptoms when making a diagnosis of Mycoplasma pneumoniae infection. This test is not intended to replace culture, and should not be used as the sole basis for diagnosis.
False positive results may occur with sera from patients with other Mycoplasma infections, pancreatitis, bacterial meningitis and other acute inflammatory disease. Cross-reactivity of this assay with antibodies to the above disease states has not been determined. Epidemiology of case, symptoms and other laboratory tests can help in differentiating these conditions from Mycoplasma pneumoniae.
Icteric, lipemic, hemolyzed or heat inactivated sera may cause erroneous results and should be avoided.
A negative antibody result does not rule out Mycoplasma pneumoniae infection. False negative results may occur when samples are drawn too early after onset. Production of detectable antibody levels may be delayed. Some patients may never generate detectable antibody levels. Patients with symptoms suggestive of Mycoplasma pneumoniae infection with negative test results should be retested in 4-6 weeks using paired sera analysis.
Mycoplasma pneumoniae infection can have a long incubation period, thus elevated antibody titers in the acute specimen are common, and reinfection may occur. Therefore, seroconversion (negative to positive) is unusual.
A positive single serum result only indicates prior exposure to Mycoplasma pneumoniae. The antibody level in a single specimen does not have significance for disease severity. The presence or absence of antibody cannot be used to determine the success or failure of antibiotic therapy.
Screening of the general population should not be performed. Testing should only be performed when clinical symptoms are present or exposure is suspected.
The results of ELISA immunoassays performed on serum from immunocompromised patients must be interpreted with caution.

## EXPECTED RESULTS

It is reported that the percentage of pneumonias caused by M. pneumoniae is 10-33% in the general population, 35-74% in children (5-19 years old), 27-52% in college students, 8-54% in military recruits and 7-17% in civilian adults (12).
An increase of IgG relative to IgM antibodies occurs with time after onset of illness. Also, adults respond with higher IgG antibody ratios than do children (2). IgM titers are significant in a high percentage of patients at admission (1). Seventy-five normal sera from asymptomatic blood donors were tested and the reported IgG activities (U/mL) are shown in Figure 1.

**Figure 1**




## PERFORMANCE CHARACTERISTICS

Reference results (CF or IAHA method) are classified as: 1) greater than a four–fold titer increase, 2) single value above 1:1024 titer, 3) falling titer greater than four-fold, or 4) no significant change. Paired samples were not selected by the sites, but were frozen and retrospectively evaluated.
Note: The majority of samples are from reference laboratories that may receive selected samples. Since assay performance depends on the prevalence of disease in a population, users may not see the same performance in their laboratory.

## SENSITIVITY

Table 5 compares reference method results to ImmunoWELL IgG results, not taking into account ImmunoWELL IgM test results. Combining the CF and IAHA rise, fall and greater than or equal to 1024 titer results as significant positives, the relative sensitivity is 81% (67-90%). The relative specificity is 100% (66-100%).

**Table 5: Relative Performance (Only ImmunoWELL IgG Results)**

| ImmunoWELL IgG Ratio Result | CF and IAHA Combined | | | | |
|---|---|---|---|---|---|
| | Rise | Fall | 1024 Titer | No Change | Total |
| Agree | 34 | 6 | 2 | 0 | 42 |
| Disagree | 2 | 4 | 4 | 9 | 19 |
| Total | 36 | 10 | 6 | 9 | 61 |

Using the same pairs evaluated in Table 5, pairs showing an ImmunoWELL IgM positive result are excluded from IgG consideration. The relative performance of ImmunoWELL IgG to CF and IAHA results is shown in Table 6. There is 75% (43-95%) agreement with traditional serology testing for positive pairs and 100% (66-100%) agreement for negative pairs.

**Table 6: Relative Performance (Without IgM Positives)**

| ImmunoWELL IgG Ratio Results (without IgM positive pairs) | CF and IAHA Combined | | | | |
|---|---|---|---|---|---|
| | Rise | Fall | 1024 Titer | No Change | Total |
| Agree | 7 | 1 | 0 | 8 | 16 |
| Disagree | 2 | 1 | 0 | 0 | 3 |
| Total | 9 | 2 | 0 | 8 | 19 |

Overall relative performance following both ImmunoWELL IgM and IgG package insert guidelines is shown in Table 7. The sensitivity is 96% (87-100%) and specificity is 89% (52-100%).

**Table 7: Relative Performance (IgM and IgG Results)**

| ImmunoWELL IgM and IgG Ratio Results | CF and IAHA Combined | | | | |
|---|---|---|---|---|---|
| | Rise | Fall | 1024 Titer | No Change | Total |
| Agree | 35 | 9 | 6 | 8 | 58 |
| Disagree | 1 | 1 | 0 | 1 | 3 |
| Total | 36 | 10 | 6 | 9 | 61 |

Performance of standard serology methods using both ImmunoWELL IgM and IgG results shows a relative sensitivity of 95% (58/61) and a relative specificity of 89% (8/9). Since the above studies represent a selected population, no positive or negative predictive values may be calculated.

*Note: Please be advised that "relative" refers to the comparison of this assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease or infection.*

## SPECIFICITY

Forty-five sera collected from asymptomatic blood donors were used to assess specificity. Two separate dilutions to mimic acute and convalescent preparations were made from each sample. The two dilutions (i.e., acute and convalescent) were tested in ImmunoWELL and the ratios were calculated as described in the INTERPRETATION section. In no case was a significant titer rise observed. Therefore the specificity is 100% (92–100%).

## REPRODUCIBILITY

Twenty assay runs, testing samples in duplicate, using common reagents on twenty different days were made at GenBio. The assay's results are shown in Table 8.

Caution: EIA precision may vary between laboratories.

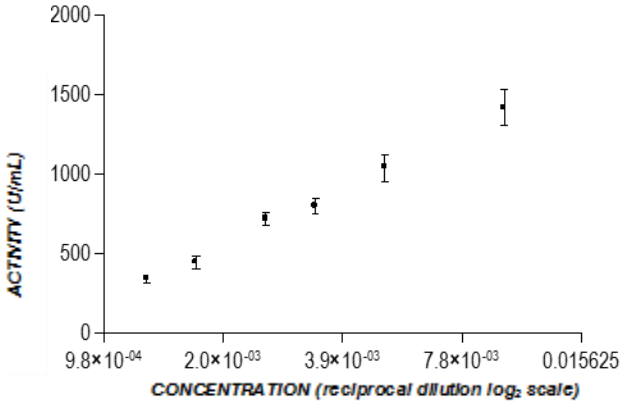
**Table 8: Assay Precision**

| Ratio | Within Run Variation (% CV) | Between Day Variation (% CV) |
|---|---|---|
| 5.8 | 11% | 29% |
| 2.6 | 8% | 19% |
| 2.1 | 10% | 23% |

## ASSAY LINEARITY

Triplicate, two-fold serial dilutions of one positive serum were tested in duplicate. The results are shown in Figure 2. The r2 is 0.92 and the linear regression runs test supports linearity from 200 to 1500 units.

**Figure 2: Assay Linearity**




## BIBLIOGRAPHY


1. **Ali, N H, et al.** The Clinical Spectrum and Diagnosis of Mycoplasma pneumoniae Infections. *Q J Med.* 1986, Vol. 58, p. 227.
2. **Foy, H M.** Mycoplasma Pneumoniae. [book auth.] A S Evans. *Bacterial Infections of Humans: Epidemiology and Control.* New York : Plenum Publishing, 1982, p. 345.
3. **Foy, H M, et al.** Long Term Epidemiology of Infections with Mycoplasma pneumoniae. *J Infect Dis.* 1979, Vol. 139, p. 681.
4. **Cherry, J D, Hurwitz, E S and Welliver, R C.** Mycoplasma pneumoniae Infections and Exanthems. *J Pediat.* 1975, Vol. 87, p. 369.
5. **Levy, M and Shear, N H.** Mycoplasma pneumoniae Infections and Steven–Johnson Syndrome: Report of Eight Cases and Review of the Literature. *Clin Pediatr.* 1991, Vol. 30, p. 42.
6. **Sillis, M.** The Limitations of IgM Assays in the Serological Diagnosis of Mycoplasma pneumoniae Infections. *J Med Microbiol.* 1990, Vol. 33, p. 253.
7. **US Centers for Disease Control.** *Manual Guide – Safety Management No. CDC–22 Decontamination of Laboratory Sink Drains to Remove Azide Salts.* Atlanta : Centers for Disease Control, 1976.
8. —. *HHS Publication No. (CDC) 93-8395, 3rd ed: Biosafety in Microbiological and Biomedical Laboratories.* Washington DC : US Government Printing Office, 1993.
9. **US Department of Labor, Occupational Safety and Health Administration.** *29 CFR Part 1910.1030, Occupational safety and health standards, bloodborne pathogens.*
10. **US Department of Health and Human Services.** *HHS Publication No. (CDC) 21-11: Biosafety in Microbiological and Biomedical Laboratories. 5th ed.* Washington DC : US Government Printing Office, 2009.
11. **World Health Organization.** *Laboratory Biosafety Manual 3rd ed.* Geneva : World Health Organization, 1991.
12. **Keitel, W A and Couch, R B.** Mycoplasma pneumonia: In the differential all year. *J Resp Dis.* 1985, 119.

QUICK REFERENCE PROCEDURE

## *IMMUNOWELL* MYCOPLASMA IGG

- Prepare Wash Buffer from Wash Concentrate.
- Dilute each control and specimen 1:100 in Specimen Diluent.
- Add 100 µL of Specimen Diluent into the first well as a substrate blank.
- Pipet 100 µL of the prediluted calibrator, controls and specimens into coated microwells and incubate 60 min at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Pipet 100 µL of Conjugate into microwells and incubate 30 min at room temperature.
- Aspirate microwells and wash microwells three times with Wash Buffer.
- Prepare fresh Color Developer.
- Pipet 100 µL of Color Developer into microwells and incubate 30 min at room temperature
- Pipet 100 µL of Stop Solution into microwells and read results at 405 nm.

To place an order for ImmunoWELL products, contact your local distributor, or call GenBio directly for the distributor nearest you and for additional product information.
For assistance, please call toll-free 800-288-4368.
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