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Product Information

***Mycoplasma Pneumoniae* IgG ELISA**

Catalog Number **SE120095**
Storage Temperature 2–8 °C

TECHNICAL BULLETIN

Product Description

Mycoplasma pneumoniae (*M. pneumoniae*) is a pathogen with a spectrum of clinical presentations ranging from asymptomatic to pronounced pneumonia. Symptoms start from 6–32 days after exposure with headache, malaise, cough, sore throat, and fever. The illness can last from a few days to a month or more. Detection by ELISA of *M. pneumoniae* IgM antibodies or demonstration of a significant increase of specific IgG antibodies is strong evidence for recent infection in the appropriate clinical setting. Specific IgM antibodies typically increase significantly 1 week after clinical onset and specific IgG levels rise in the second week. *M. pneumoniae* IgM can, however, persist for more than two years after infection, and therefore, detection of specific IgM does not accurately indicate the time of infection. Primary infection and reinfection may be distinguished by the presence of elevated specific IgA and of specific IgM in primary infections and by the presence of elevated specific IgA in the absence of specific IgM in reinfections. In general, the absence of specific IgM in serum collected 10–20 days after onset is strong evidence against primary pneumonia due to *M. pneumoniae*.

The *Mycoplasma pneumoniae* IgG ELISA kit is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to *M. pneumoniae* in human serum or plasma. Diluted serum is added to wells coated with purified antigen. IgG specific antibody, if present, binds to the antigen. All unbound materials are washed away and the Enzyme Conjugate is added to bind to the antibody-antigen complex, if present. Excess Enzyme Conjugate is washed off and Substrate is added. The plate is incubated to allow the oxidation of the substrate by the enzyme. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample.

Components

Materials Provided	96 Tests
Microwells coated with *M. pneumoniae* antigen	12 x 8 x 1
Sample Diluent: 1 bottle (ready to use)	22 mL
Calibrator: 1 vial (ready to use)	1 mL
Positive Control: 1 vial (ready to use)	1 mL
Negative Control: 1 vial (ready to use)	1 mL
Enzyme conjugate: 1 bottle (ready to use)	12 mL
TMB Substrate: 1 bottle (ready to use)	12 mL
Stop Solution: 1 bottle (ready to use)	12 mL
Wash concentrate 20x: 1 bottle	25 mL

Reagents and Equipment Required but Not Provided.

1. Distilled or deionized water
2. Precision pipettes
3. Disposable pipette tips
4. ELISA reader capable of reading absorbance at 450 nm
5. Absorbent paper or paper towel
6. Graph paper

Precautions and Disclaimer

This product is for R&D use only, not for drug, household or other uses. Please consult the Safety Data Sheet for information regarding hazards and safe handling practices.

Preparation Instructions

Sample Preparation

1. Collect blood specimens and separate the serum.
2. Specimens may be refrigerated at 2–8 °C for up to seven days or frozen for up to six months. Avoid repetitive freezing and thawing

20x Wash Buffer Concentrate
Prepare 1x Wash buffer by adding the contents of the bottle (25 mL, 20x) to 475 mL of distilled or deionized water.

Storage/Stability
Store the kit at 2–8 °C.

Procedure
Notes: The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.

Optimal results will be obtained by strict adherence to the test protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.

The test run may be considered valid provided the following criteria are met:

1. If the O.D. of the Calibrator is >0.250.
2. The Ab index for Negative control should be <0.9.
3. The Ab index for Positive control should be >1.2.

Bring all specimens and kit reagents to room temperature (18–26 °C) and gently mix.

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 21-fold dilution of test samples, by adding 10 µl of the sample to 200 µL of Sample Diluent. Mix well.
3. Dispense 100 µL of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µL of Sample Diluent in 1A well position. Tap the holder to remove air bubbles from the liquid and mix well. Incubate for 20 minutes at room temperature.
4. Remove liquid from all wells. Wash wells three times with 300 µL of 1x wash buffer. Blot on absorbent paper or paper towel.
5. Dispense 100 µL of Enzyme Conjugate to each well and incubate for 20 minutes at room temperature.
6. Remove Enzyme Conjugate from all wells. Wash wells three times with 300 µL of 1x Wash Buffer. Blot on absorbent paper or paper towel.
7. Dispense 100 µL of TMB Substrate and incubate for 10 minutes at room temperature.
8. Add 100 µL of Stop Solution.
9. Read O.D. at 450 nm using ELISA reader within 15 minutes. A dual wavelength is recommended with reference filter of 600–650 nm.

Results

Calculations

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure the value is checked on every kit.
2. Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
3. Calculate the Ab (Antibody) Index of each determination by dividing the O.D. value of each sample by cut-off value.

Example of typical results:
Calibrator mean OD = 0.8
Calibrator Factor (CF) = 0.5
Cut-off Value = 0.8 x 0.5 = 0.400
Positive control O.D. = 1.2
Ab Index = 1.2/0.4 = 3
Patient sample O.D. = 1.6
Ab Index = 1.6/0.4 = 4.0

Note: Lipemic or hemolyzed samples may cause erroneous results.

Interpretation

The following is intended as a guide to interpretation of *M. pneumoniae* IgG antibody index (Ab Index) test results; each laboratory is encouraged to establish its own criteria for test interpretation based on sample populations encountered.

<0.9 – No detectable antibody to *M. pneumoniae* IgG by ELISA

0.9–1.1 – Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 – Detectable antibody to *M. pneumoniae* IgG by ELISA

References

1. Quinn, T.C., Diagnosis of atypical pneumonias: *Legionella*, *Chlamydia*, and *Mycoplasma* infections. Ann. Intern. Med., 1996;124:591-4.
2. Cimolai, N., and Cheong, A.C.H., An assessment of a new diagnostic indirect enzyme immunoassay for the detection of anti-*Mycoplasma pneumoniae* IgM. Am. J. Clin. Pathol., 1996;105:205-9.
3. Shearman, M.J. et al., *Mycoplasma pneumoniae* infection: early diagnosis by detection of specific IgM by immunofluorescence. Br. J. Biomed. Sci., 1993;50:305-8.
4. Lee, S.H. et al., Comparative studies of three serologic methods for the measurement of Mycoplasma pneumoniae antibodies. Am. J. Clin. Pathol., 1989;92:342-7.
5. Kenny, G.E. et al., Diagnosis of *Mycoplasma pneumoniae* pneumonia: sensitivities and specificities of serology with lipid antigen and isolation of the organism on soy peptone medium for identification of infections. J. Clin. Microbiol., 1990;28:2087- 93.
6. Aubert, G. et al., Evaluation of five commercial tests: complement fixation, microparticle agglutination, indirect immunofluorescence, enzyme-linked immunosorbent assay and latex agglutination, in comparison to immunoblotting for *Mycoplasma pneumoniae* serology. Ann. Biol. Clin., 1992;50:593
7. Kok, T. et al., Routine diagnosis of seven respiratory viruses and *Mycoplasma pneumoniae* by enzyme immunoassay. J. Virol. Methods, 1994; 50:87-100.
8. Kleemola, M. et al., Evaluation of an antigen-capture enzyme immunoassay for rapid diagnosis of *Mycoplasma pneumoniae* infection. Eur. J. Clin. Microbiol. Infect. Dis., 1993;12:872-5.

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