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Product Information

Mycoplasma pneumoniae IgG ELISA kit

Catalog Number: EA100955
Storage Temperature: 2 – 8°C

Instruction for Use

Intended Use

The *Mycoplasma pneumoniae (M. pneumoniae)* IgG ELISA test system is an enzyme linked immunosorbent assay (ELISA) for the detection of IgG class antibodies to *M. pneumoniae* in human serum or plasma.

Background

Mycoplasma pneumoniae is a pathogen with spectrum of clinical presentations ranging from asymptomatic to pronounced pneumonia. Symptoms start from 6 to 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*.

Principle of the Test

Diluted patient 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 hydrolysis 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
1. Microwells coated with *M. pneumoniae* antigen	12x8x1
2. Sample Diluent: 1 bottle (ready to use)	22 ml
3. Calibrator: 1 Vial (ready to use)	1ml
4. Positive Control: 1 vial (ready to use)	1ml
5. Negative Control: 1 vial (ready to use)	1ml

6. Enzyme conjugate: 1 bottle (ready to use)	12ml
7. TMB Substrate: 1 bottle (ready to use)	12ml
8. Stop Solution: 1 bottle (ready to use)	12ml
9. Wash concentrate 20X: 1 bottle	25ml

Materials 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 450nm
5. Absorbance paper or paper towel

Disclaimer

This product is for research use only and not intended for diagnostic procedures.

Specimen Collection and 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.

Reagent Preparation

1. Prepare 1X Wash buffer by adding Wash Concentrate (25 ml, 20X) to 475 ml of distilled or deionized water. Store at room temperature (18-26°C).

Assay Procedure

- Before proceeding with the assay, bring all reagents, serum references and controls to room temperature (18-26°C). Gently mix all reagents before use
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed
- It is recommended that standards, control and serum samples be run in duplicate
- Do not use sodium azide as preservative. Sodium azide inhibits HRP enzyme activities

1. Place the desired number of coated strips into the holder.
2. Negative control, positive control, and calibrator are ready to use. Prepare 1:21 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 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 absorbance paper or paper towel.
5. Dispense 100 µl l 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 absorbance paper or paper towel.
7. Dispense 100 µl of TMB substrate and incubate for 10 minutes at room temperature. Add 100 µl of stop solution.

8. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm

Calculation of Results

1. Check Calibrator Factor (CF) value on the calibrator bottle. This value might vary from lot to lot. Make sure you check the value 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 a Standard Curve

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

Quality Control

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

1. If the O.D. of the Calibrator should be greater than 0.250.
2. The Ab index for Negative control should be less than 0.9.
3. The Ab index for Positive control should be greater than 1.2.

Interpretation

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

- **Antibody Index Interpretation**
  - <0.9 No detectable antibody to *M. pneumoniae* IgG by ELISA
  - 0.9-1.1 Borderline positive. Follow-up testing is recommended if clinically indicated.
  - >1.1 Detectable antibody to *M. pneumoniae* IgG by ELISA

References

1. Quinn TC. Diagnosis of atypical pneumonias: Legionella, Chlamydia, and Mycoplasma infections. Ann Intern Med 1996;124:591-4.
2. Cimolai N, Cheong ACH. 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.
4. Shearman MJ, Cubie HA, Inglis JM. Mycoplasma pneumoniae infection: early diagnosis by detection of specific IgM by immunofluorescence. Br J Biomed Sci 1993;50:305-8.
3. Lee SH, Charoenying S, Brennan T, Markowski M, Mayo DR. Comparative studies of three serologic methods for the measurement of Mycoplasma pneumoniae antibodies. Am J Clin Pathol 1989;92:342-7.
4. Kenny GE, Kaiser GG, Cooney MK, Foy HM. 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.

5. Aubert G, Pozzetto B, Gaudin OG, Hafid J, Mbida AD, Ros A. 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
6. Kok T, Mickan LD, Burrell CJ. Routine diagnosis of seven respiratory viruses and Mycoplasma pneumoniae by enzyme immunoassay. J Virol Methods 1994;50:87-100.
7. Kleemola M, Räty R, Karjalainen J, Schuy W, Gerstenecker B, Jacobs E. 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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