Chlamydia Pneumoniae IgG ELISA Kit

Catalog Number KA0956

96 assays

Version: 03

Intended for research use only

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Introduction

Intended Use

The Chlamydia Pneumoniae IgG ELISA Kit is intended for the detection of IgG antibody to *C. Pneumoniae* in human serum or plasma.

Background

Chlamydia pneumoniae, the third recognized of five possible species of Chlamydia (trachomatis, psittaci, pneumoniae, pecorum and an as-yet-unnamed species) was formerly known as Chlamydia spp. Strain TWAR. This respiratory pathogen which causes acute respiratory disease, pneumonia and pharyngitis is often isolated from patients with otitis media with effusion, pneumonia with pleural effusion and in asymptomatic respiratory tract infections. *C. pneuomoniae* causes up to 10% of community-acquired pneumonia cases and it is also a risk factor for coronary heart disease and Guillain-Barré syndrome. Seroprevalence of *C.pneumoniae* among children is low and increases sharply in teenagers, continues to increase until middle age, and remains high (>50%) into old age, suggesting that most people have more than one *C.pneumoniae* infection during their lifetime. Primary Chlamydia infection is characterized by a predominant IgM response within 2 to 4 weeks and a delayed IgG and IgA response within 6 to 8 weeks. After acute *C.pneumoniae* infection, IgM antibodies are usually lost within 2 to 6 months IgG antibody titers rise and usually decrease slowly; whereas IgA antibodies tend to disappear rapidly. When primary Chlamydia infection is suspected, the detection of IgM is highly diagnostic. In reinfection, IgM level may be rarely detected while IgG and IgA levels rise quickly, often in one to two weeks. IgA antibodies have shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment and eradication of the Chlamydia infections.

Principle of the Assay

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 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.

General Information

Materials Supplied

List of component

Component	Amount
Microwells coated with *C. pneumoniae* antigen	96 (8x12) wells
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

Storage Instruction

- ✓ Store the kit at 2-8°C.
- ✓ Keep microwells sealed in a dry bag with desiccants.
- ✓ The reagents are stable until expiration of the kit.
- ✓ Do not expose test reagents to heat, sun, or strong light

Materials Required but Not Supplied

- ✓ Distilled or deionized water
- ✓ Precision pipettes
- ✓ Disposable pipette tips
- ✓ ELISA reader capable of reading absorbance at 450 nm
- ✓ Absorbance paper or paper towel
- ✓ Graph paper

Precautions for Use

- ✓ Precautions
- • Potential biohazardous materials:

  The calibrator and controls contain human source components which have been tested and found non-reactive for hepatitis B surface antigen as well as HIV antibody with FDA licensed reagents. However, as there is no test method that can offer complete assurance that HIV, Hepatitis B virus or

other infectious agents are absent. These reagents should be handled at the Biosafety Level 2, as recommended in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories." 1984.

- This test kit is designed for Research Use Only.
- Optimal results will be obtained by strict adherence to this protocol. Precise pipetting as well as following the exact time and temperature requirements is essential.
- Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
- The components in this kit are intended for use as an integral unit. The components of different lots should not be mixed.
- Control sera and sample diluent contain preserved with sodium azide. Sodium szide may react with lead and cooper plumbing to form explosive metal azide. On disposal, flush with a large volume of water.

✓ Limitation of the test
- Lipemic or hemolyzed samples may cause erroneous results.

Assay Protocol

Reagent Preparation

Prepare 1X Wash buffer by adding the contents of the bottle (25 mL, 20X) to 475 mL of distilled or deionized water. Store at room temperature (20-25°C).

Sample Preparation

- ✓ Collect blood specimens and separate the serum.
- ✓ 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.

Assay Procedure

Bring all specimens and kit reagents to room temperature (20-25°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 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 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.
8. Add 100 µL of stop solution.
9. Read O.D. at 450 nm using ELISA reader within 15 min. A dual wavelength is recommended with reference filter of 600-650 nm.

Data Analysis

Calculation of Results

The standard curve is constructed as follows:
- ✓ 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.
- ✓ Calculate the cut-off value: Calibrator OD x Calibrator Factor (CF).
- ✓ 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

  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. 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 fall within the range specified on the COA/label.

- Interpretation

  The following is intended as a guide to interpretation of this *C. 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 *Chlamydia pneumonia* IgG by ELISA.

  0.9-1.1   Borderline positive. Follow-up testing is recommend.

  >1.1   Detectable antibody to *Chlamydia pneumoniae* IgG by ELISA.

Performance Characteristics

- Sensitivity and Specificity

  86 sera were tested by this *Chlamydia Pneumoniae* IgG ELISA Kit and a reference ELISA method. 62 sera were positive and 19 were negative by both methods (94% agreement). The results are summarized below:

		C. pneumoniae IgG ELISA		
		+	−	Total
Reference ELISA Kit	+	62	3	65
	−	2	19	21
	Total	72	22	86

- Precision
- ✓ Intra-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	16	1.51	0.094	6.2
2	16	1.24	0.081	6.5
3	16	0.19	0.018	9.5

- ✓ Inter-Assay Study

Serum	No. of Replicates	Mean	Standard Deviation	Coefficient of Variation %
1	10	1.67	0.16	9.5
2	10	1.22	0.114	9.3
3	10	0.023	0.027	11.7

Resource

Reference

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3. Bourke SJ, Lightfoot NF. Chlamydia pneumoniae: defining the clinical spectrum of infection requires precise laboratory diagnosis. Thorax 1995;50(Suppl 1):543-8.

4. Hammerschlag MR, Chirgwin K, Roblin PM, et al. Persistent infection with Chlamydia pneumoniae following acute respiratory illness. Clin Infect Dis 1992;14:178-82.

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6. Cook PJ, Honeybourne D. Chlamydia pneumoniae. J Antimicrobial Chemother 1994;34:859-73.

7. Nystrom-Rosander C, Thelin S, Hjelm E, et al. High incidence of Chlamydia pneumoniae in sclerotic heart valves of patients undergoing aortic valve replacement. Scan J Infect Dis 1997;29:361-5

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												