Chlamydia pneumoniae IgA ELISA Kit

Catalog Number KA2081
96 assay
Version: 01

Intend for research use only
Introduction and Background

A. Intended Use

The *Chlamydia pneumoniae* IgA ELISA Kit is intended for the detection of IgA antibodies specific to *Chlamydia pneumoniae* in human serum. *Chlamydia pneumoniae* IgA ELISA Kit is a qualitative Enzyme Linked Immunosorbent Assay (ELISA) which is used as an aid in the detection of *Chlamydia pneumoniae* infection. *Chlamydia pneumoniae* IgA ELISA Kit is a new configuration of ELISA test which presents advantageous features: incubations at ambient temperature; short test duration; and utilizing ready-to-use conjugate.

B. Introduction

*Chlamydia pneumoniae* (TWAR183) is an emerging infectious agent with a spectrum of clinical manifestations, including upper and lower respiratory tract infections (1). The majority of *C. pneumoniae* infections are mild and asymptomatic yet, may cause serious diseases, such as pharyngtitis, sinusitis, acute bronchitis and community acquired pneumonia. Undetected and untreated infection may lead to prolonged and persistent disease. Recent data indicates a possible association between *C. pneumonia* infection and chronic diseases (2). Seroprevalence of *C. pneumoniae* among children is low but increases sharply until middle age, where after it remains high (>50%).

Difficulties in sample collection and inaccessibility of the infected site seriously affect the usefulness of direct detection methods. Therefore, serological testing is routinely used and serves as a non-invasive tool in identification of both distal and chronic chlamydial infections (3), where direct detection methods are rarely efficient (4). In addition, the presence of certain antibody types may also indicate the state of the disease.

Primary chlamydial 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 (5), IgG antibody titers usually decrease slowly; whereas IgA antibodies tend to disappear rapidly (6). When primary chlamydial infection is suspected, the detection of IgM is highly diagnostic (7). However, in recurrent or chronic infections the prevalence of IgM is low and therefore absence of IgM does not necessarily exclude on-going infection.

In reinfection, IgG and IgA levels rise quickly, often in one to two weeks (8).

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 (3). The persistence of elevated IgA antibody titers is generally considered as a sign of chronic infection (6).

IgG antibodies persist for long periods and decline very slowly. Therefore, the presence of IgG antibodies is mainly indicative of a chlamydia infection at an undetermined time. However, a four-fold rise in IgG or high levels of IgG antibodies may indicate an on-going chronic infection.

*Chlamydia pneumoniae* IgM ELISA Kit is an ELISA based assay in which purified elementary bodies of *C. pneumoniae* (TWAR-183) are used as antigens to detect the antibody response in humans. For complete detection of current, chronic or past infections, it is recommended to determine IgG, IgM and IgA antibodies to *C. pneumoniae*. 
C. Principle of the Test

- Plates are coated with *C. pneumoniae* specific peptides.

- Serum to be tested is diluted and incubated with the pre-coated plate 30 minutes at Room Temperature (RT). In this step *C. trachomatis* specific antibodies are bound to the immobilized *C. pneumoniae* specific peptides.

- Non-specific antibodies are removed by washing.

- Anti-human IgM conjugated to horseradish peroxidase (HRP) is added and incubated 30 minutes at Room Temperature. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.

- Unbound conjugate is removed by washing.

- Upon the addition of TMB substrate, the substrate is hydrolyzed by the peroxidase, yielding a blue solution of the reduced substrate.

- Upon the addition of the stop solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450/620 nm.

- The absorbance is proportional to the amount of the specific antibodies which are bound to the immobilized *C. pneumoniae* specific antigens.

D. Warning and Precautions

1. This kit contains human sera and found to be negative for HBV antigen, and for antibodies to HCV and to HIV 1 & 2. Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be handled as potentially infectious serum or blood, according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories", 1988.

2. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.

3. Diluted sulfuric acid (1M H₂SO₄) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.

4. Each vial of HRP Conjugate can only be used twice. Please discard after the second time.

5. All the components of this kit have been calibrated and tested by lot. It is not recommended to mix components from different lots since it might affect the results.
E. Summary of Procedure

Manual procedure:

Wells of microtiter plate coated with *C. pneumoniae* antigens
↓
Add 2 x 50μl of Cut Off Control
Add 1 x 50μl each of Negative Control, Positive Control and diluted specimens
↓
Cover plate and incubate 30 minutes at Room Temperature.
↓
Wash 5 times with Wash Buffer
↓
Add 50μl of Ready to Use HRP-Conjugate
↓
Cover plate and incubate 30 minutes at Room Temperature
↓
Wash 5 times with Wash buffer
↓
Add 100μl of TMB-Substrate
↓
Cover plate and incubate 30min at room temperature
↓
Add 100μl of Stop Solution
↓
Read absorbance at 450/620nm
↓
Calculate and interpret results
Automation procedure:

Wells of microtiter plate coated with *C. pneumoniae* antigens

↓

Add 2 x 100μl of Cut Off Control

Add 1 x 100μl each of Negative Control, Positive Control and diluted specimens

↓

Cover plate and incubate 20 minutes at Room Temperature.

↓

Wash 5 times with Wash Buffer

↓

Add 50μl of Ready to Use HRP-Conjugate

↓

Incubate 30 minutes at Room Temperature

↓

Wash 5 times with Wash buffer

↓

Add 100μl of TMB-Substrate

↓

Incubate 30min at room temperature

↓

Add 100μl of Stop Solution

↓

Read absorbance at 450/620nm

↓

Calculate and interpret results
Material and Method

F. Kit contents: for Manual use/Automated use

1. *C. pneumoniae* antigen-coated microtiter plate: 96 break-apart wells (8x12) coated with *C. pneumoniae* specific peptides, packed in an aluminum pouch containing a desiccant card.

   1 Plate


   1 bottle, 100 ml

3. Serum Diluent-RT (Blue): A ready to use anti human IgG in buffer solution. Contains less than 0.05% proclin as a preservative.

   1 bottle, 30 ml

4. Ready to Use HRP-Conjugate (Green): Horseradish Peroxidase (HRP) conjugated anti-human IgM (μ chain specific). Contains less than 0.05% Proclin as a preservative.

   2 bottles, 4 ml each

5. Cut Off Control: A ready to use *C. pneumoniae* IgA serum used for cut off determination. Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.

   1 Vial, 2.5ml

6. Negative Control: A ready to use *C. pneumoniae* IgA negative human serum. Contains less than 0.05% Proclin and less than 0.1% Sodium Azide as preservatives.

   1 Vial, 2 ml

7. Positive Control: A ready to use *C. pneumoniae* IgA positive human serum. Contains less than 0.05% Proclin and less than 0.1% Sodium Azide as preservatives.

   1 Vial, 2 ml

8. TMB-Substrate: A ready to use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.

   1 Bottle, 14 ml

9. Stop Solution: A ready to use solution. Contains 1M H₂SO₄

   1 Bottle, 15 ml

10. Plate Cover: 1 unit

G. Materials Required But Not Supplied

- Clean test tubes for dilution of patients’ sera.
- Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000μl ranges) and disposable tips.
- One liter volumetric flask.
- One 50ml volumetric cylinder.
- Wash bottle.
- Absorbent paper.
- Vortex mixer.
ELISA-reader with 450/620nm filter.
Distilled or double deionized water.

H. Storage and Shelf-Life of Reagents
1. All the reagents supplied should be stored at 2-8°C. The unopened reagent vials are stable until the expiration date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**
2. Once the kit is opened, its shelf life is 90 days.
3. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Redissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to 21 days.

I. Serum Collection and Storage
Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.
Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If a longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

J. Test Procedure for Manual Use

- Preparation of Reagents
1. Bring all components and clinical specimens to be tested to room temperature. Mix well the Cut Off Control, Negative Control, Positive Control and the clinical specimens before use.
2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: two wells of Cut Off Control, and one well of each Negative Control and Positive Control.
3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.
4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

- Incubation of sera samples and controls
5. Dilute each patient serum 1/110 with the supplied Serum Diluent-RT as follows: Add 10μl of patient serum to 1090μl of Serum Diluent.
6. Pipette Cut-Off Control in duplicate: 50μl into each well. Pipette 50μl of each: Negative Control, Positive
control, and 1/110 diluted sera into separate wells of the test strip.

7. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22ºC-28ºC).

8. Discard the liquid content of the wells.

9. **Washing step:** Fill each well with Wash Buffer (300-350μL) up to the end of the well and discard the liquid, repeat this step 4 times, for a total of 5 washing steps.

10. Dry the strips and frame by gently tapping them over clean absorbent paper.

● **Incubation with Conjugate**
  
  *Each vial of HRP Conjugate can only be used twice.*

11. Pipette 50μl of ready to use HRP Conjugate into each well.

12. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22ºC-28ºC).

13. Discard the liquid content and wash as described in steps 9-10.

● **Incubation with TMB - Substrate**

14. Dispense 100μl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature (22ºC-28ºC) for 30 minutes.

15. Stop the reaction by adding 100μl of Stop Solution (1M H₂SO₄) to each well.

● **Determination of Results**

16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

*Note: Any air bubbles should be removed before reading. The bottom of the ELISA plate should be carefully wiped.*

K. **Test Procedure for Automated Use**

*The vials and reagents' volume have been adapted for automation applications.*

● **Preparation of Reagents**

1. Bring all components and the clinical specimens to be tested to room temperature. Mix well the Cut-Off Control, Negative Control, Positive Control and the clinical specimens before use.

2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: Two wells of Cut-Off Control and one well of each Negative Control and Positive Control.

3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.

4. Dilute the Concentrated Wash Buffer 1/20 with double deionized or distilled water. For example, in order to prepare one liter of wash buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

● **Incubation of sera samples and controls**

5. Dilute each patient serum 1/110 as follows: Dispense 1090μl of Serum Diluent-RT to each sample’s tube. Add 10μl patient serum to each sample’s tube.

6. Pipette Cut-Off Control in duplicate: 100μl into each well. Pipette 100μl of each: Negative Control, Positive control, and 1/110 diluted sera into separate wells of the test strip.
7. Incubate for 20 minutes at room temperature (22-28°C).
8. **Eliminate assay drift caused by this operation.**
9. **Washing step:** Perform 5 X 500μl wash cycles using Wash Buffer.
   - **Incubation with conjugate**
     
     Each vial of HRP Conjugate can only be used twice
11. Dispense 50μl of Ready-to-Use HRP-conjugate into each well.
12. Incubate for 30 minutes at room temperature (22-28°C).
13. Wash as described in steps 9-10.
   - **Incubation with TMB – Substrate**
14. Dispense 100μl TMB-Substrate into each well and incubate at room temperature (22-28°C) for 30 minutes in the dark.
15. Stop the reaction by adding 100μl of Stop Solution (1M H₂SO₄) to each well.
   - **Determination of Results**
16. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction

*Please note that each automation machine has specific technical commands. Please implement Abnova’s automation procedure for this kit on the operation protocol of your automation machine.*
Performance Characteristics

L. Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. O.D. Positive Control \( \geq 0.8 \)
2. Ratio O.D. Positive Control / O.D. Cut Off Control > 2
3. O.D. negative control < 0.3

M. Calculation of Test Results

1. The average absorbance value of the Cut off serum run in duplicate should be calculated.
2. In order to normalize the results obtained in different tests, the cut off index (COI) is calculated according to the following formula:

   \[ \text{COI} = \frac{\text{OD of the Serum Sample}}{\text{OD Average of Cut Off Control}} \]

N. Interpretation of Results

Table 1

<table>
<thead>
<tr>
<th>COI</th>
<th>Results</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;1.0</td>
<td>Negative</td>
<td>No indication of current infection by <em>C. pneumoniae</em></td>
</tr>
<tr>
<td></td>
<td>no detectable IgA antibodies</td>
<td></td>
</tr>
<tr>
<td>1-1.1</td>
<td>Borderline</td>
<td>May indicate possible exposure to <em>C. pneumoniae</em>.</td>
</tr>
<tr>
<td></td>
<td>low level of IgA antibodies</td>
<td>Second sample testing required after 2-4 weeks(^1)</td>
</tr>
<tr>
<td>&gt;1.1</td>
<td>Positive</td>
<td>May indicate current or past infection by <em>C. pneumoniae</em>(^2)</td>
</tr>
<tr>
<td></td>
<td>relevant levels of IgA antibodies</td>
<td></td>
</tr>
</tbody>
</table>

1. When testing a second sample, both the first and the second sample should be tested simultaneously.
2. In order to differentiate between current or chronic infection follow up studies are recommended.

In order to achieve a more comprehensive antibodies' profile, IgM and IgG should also be tested

Table 2: Interpretation of results based on the combination of IgG, IgA and IgM antibodies.

<table>
<thead>
<tr>
<th>Levels of <em>C. pneumoniae</em> specific antibodies</th>
<th>Interpretation of Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>IgG</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative or Positive</td>
</tr>
<tr>
<td>Positive</td>
<td>Negative or Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Negative</td>
<td>Positive or Negative</td>
</tr>
</tbody>
</table>
O. Precision

Table 3: Intra-assay (within-run) precision of the *Chlamydia pneumoniae* IgA ELISA Kit is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Replicates</th>
<th>Mean Value</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
<td>1.296</td>
<td>3.92</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>0.262</td>
<td>6.54</td>
</tr>
</tbody>
</table>

Table 4: Inter-assay (between-run) precision of the *Chlamydia pneumoniae* IgA ELISA Kit is shown below:

<table>
<thead>
<tr>
<th>Sample</th>
<th>No. of Replicates</th>
<th>Mean Value</th>
<th>CV%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>12</td>
<td>1.221</td>
<td>7.3</td>
</tr>
<tr>
<td>Negative</td>
<td>12</td>
<td>0.127</td>
<td>12.3</td>
</tr>
</tbody>
</table>

P. Test Limitations

1. Samples obtained too early during primary infection may not contain detectable antibodies. If Chlamydia infection is suspected, a second sample should be obtained 2-4 weeks later and tested in parallel with the original sample.

2. Interfering substances: The use of lipemic, turbid or contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.
Reference


