Chlamydia Trachomatis IgG ELISA Kit

Catalog Number KA0959
96 assays
Version: 03

Intended for research use only
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Introduction

Intended Use

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

Background

*C. trachomatis* is an intracellular parasite pathogen that is similar in cell wall structure to gramnegative bacteria. It is the most common sexually transmitted disease (STD) in the US with more than 4 million cases reported annually. The main sites of infection include the GU tract and rectum but conjunctivitis, perihepatitis and reactive arthritis may result. The infection is often asymptomatic, making it difficult to diagnose; as many as 2/3 of infected women are asymptomatic. Women develop mucopurulent cervicitis, and irregular menstrual bleeding or abdominal pain may occur in about 40% of these women. PID is found in about 5% of women. The infection is usually symptomatic in men with dysuria and white/clear discharge occurring. Epididymitis is common. The infection incubates in 7 to 21 days and is commonly found with a second STD pathogen. IgG and IgM antibodies to *C. trachomatis* can be detected with 2-4 weeks after exposure. IgG remains positive, but the antibody level can drop overtime. ELISA can detect *C. trachomatis* IgM antibody for many months after infection.

Principle of the Assay

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.
General Information

Materials Supplied

List of component

<table>
<thead>
<tr>
<th>Component</th>
<th>Amount</th>
</tr>
</thead>
<tbody>
<tr>
<td>Microwells coated with <em>C. trachomatis</em> antigen</td>
<td>96 (8x12) wells</td>
</tr>
<tr>
<td>Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
</tr>
<tr>
<td>Calibrator: 1 Vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Positive Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Negative Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
</tr>
<tr>
<td>Wash concentrate 20X: 1 bottle</td>
<td>25 ml</td>
</tr>
</tbody>
</table>

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. Accurate and precise pipetting, as well as following the exact time and temperature requirements prescribed are 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 azide 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

✓ The test results obtained using this kit serve only as an aid to diagnosis and should be interpreted in relation to the patient's history, physical findings and other diagnostic procedures.

✓ 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 (18-26 °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 (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 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
  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. 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 C. trachomatis 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 C. trachomatis IgG by ELISA.
  0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.
  >1.1   Detectable antibody to C. trachomatis IgG by ELISA.
Performance Characteristics

- Sensitivity and Specificity
  246 patient sera were tested by this Chlamydia Trachomatis IgG ELISA and a reference ELISA method. 122 sera were positive and 118 were negative by both methods (96% agreement). The results are summarized below:

<table>
<thead>
<tr>
<th></th>
<th>C. Trachomatis IgG ELISA</th>
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<tbody>
<tr>
<td></td>
<td>+</td>
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<tr>
<td>Reference ELISA Kit</td>
<td>+</td>
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<tr>
<td></td>
<td>−</td>
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<tr>
<td><strong>Total</strong></td>
<td>125</td>
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- Precision
  ✓ Intra-Assay Study

<table>
<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
<th>Mean</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation %</th>
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<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>1.87</td>
<td>0.092</td>
<td>4.92</td>
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<tr>
<td>2</td>
<td>16</td>
<td>1.22</td>
<td>0.067</td>
<td>5.49</td>
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<tr>
<td>3</td>
<td>16</td>
<td>0.28</td>
<td>0.018</td>
<td>6.43</td>
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✓ Inter-Assay Study

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<thead>
<tr>
<th>Serum</th>
<th>No. of Replicates</th>
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<th>Coefficient of Variation %</th>
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<tbody>
<tr>
<td>1</td>
<td>10</td>
<td>1.61</td>
<td>0.142</td>
<td>4.92</td>
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<tr>
<td>2</td>
<td>10</td>
<td>1.26</td>
<td>0.107</td>
<td>5.49</td>
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<td>3</td>
<td>10</td>
<td>0.24</td>
<td>0.022</td>
<td>9.16</td>
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Reference


3. Bas S; Cunningham T; Kvien TK; Glenn as A; Melby K; Vischer TL. The value of isotype determination of serum antibodies against Chlamydia for the diagnosis of Chlamydia reactive arthritis. Br J Rheumatol 1996; 35(6):542-7


5. Verkooyen RP; Van Lent NA; Mousavi Joulandan SA; Snijder RJ; van den Bosch JM; Van Helden HP; Verbrugh HA. Diagnosis of Chlamydia pneumoniae infection in patients with chronic obstructive pulmonary disease by micro-immunofluorescence and ELISA. J Med Microbiol 1997; 46(11):959-64.
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