Chlamydia Trachomatis IgG ELISA

INTENDED USE
The Chlamydia Trachomatis IgG Kit is intended for the detection of IgG antibody to Chlamydia Trachomatis in human serum or plasma. For research use only, not for diagnostic use.

SUMMARY AND EXPLANATION
C. trachomatis is an intracellular parasite pathogen that is similar in cell wall structure to gram-negative 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, perihapatitis 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 TEST
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.

Manufactured For:
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**Antibody Index Interpretation**
- <0.9: No detectable antibody to C. trachomatis IgG by ELISA
- 0.9-1.1: Borderline positive. Follow-up testing is recommended if indicated.
- >1.1: Detectable antibody to C. trachomatis IgG by ELISA

**LIMITATIONS OF THE TEST**
1. Lipemic or hemolyzed samples may cause erroneous results.

**REFERENCES**
5. Verkooyen RP; Van Lent NA; Mousavi Joulandan SA; Snijder R; 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.
6. Verkooyen RP; Van Lent NA; Mousavi Joulandan SA; Snijder R; 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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**MATERIALS PROVIDED**
- 96 Tests

<table>
<thead>
<tr>
<th>MATERIALS PROVIDED</th>
<th>96 Tests</th>
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<tbody>
<tr>
<td>1. Microwells coated with C. trachomatis antigen</td>
<td>12x8x1</td>
</tr>
<tr>
<td>2. Sample Diluent: 1 bottle (ready to use)</td>
<td>22 ml</td>
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<tr>
<td>3. Calibrator: 1 Vial (ready to use)</td>
<td>1 ml</td>
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<tr>
<td>4. Positive Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>5. Negative Control: 1 vial (ready to use)</td>
<td>1 ml</td>
</tr>
<tr>
<td>6. Enzyme conjugate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>7. TMB Substrate: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>8. Stop Solution: 1 bottle (ready to use)</td>
<td>12 ml</td>
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<tr>
<td>9. Wash Solution concentrate 20X: 1 bottle</td>
<td>25 ml</td>
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</table>
MATERIALS 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. Absorbance paper or paper towel
6. Graph paper

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

WARNINGS AND PRECAUTIONS
1. 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, 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.
2. This kit is designed for research use only.
3. 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.
4. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit
   reagents are handled.
5. The components in this kit are intended for use as an integral unit. The components of
   different lots should not be mixed.
6. Control sera and sample diluent contain preserved with sodium azide. Sodium azide may
   react with lead and copper plumbing to form explosive metal azide. On disposal, flush with
   a large volume of water.

SPECIMEN COLLECTION AND HANDLING
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
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).

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 100μl 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.

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 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
Unknown 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.