

## Introduction and Background

### A. Overview

*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 IgA 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.

### B. Test Principle

Diluted patient serum (serum diluent contains sorbent to remove rheumatoid factor and human IgG interference) is added to wells coated with purified antigen. IgA 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 IgA specific antibody in the sample.

### C. Notice for Application of Kit

This kit has been configured for research use only and is not for diagnostic and clinical use.

### D. Application

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

## Material and Method

### A. List of component

Microwells coated with <i>C. trachomatis</i> antigen	12x8x1
Sample Diluent: 1 bottle (ready to use)	22 ml
Calibrator: 1 Vial (ready to use)	1.5 ml
Positive Control: 1 vial (ready to use)	1.5 ml
Negative Control: 1 vial (ready to use)	1.5 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

### B. Additional Required Materials But 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

### C. Stability and Storage

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.

### D. Warnings and Precautions for Users

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.

#### **E. 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.

#### **F. Reagent Preparation and Storage**

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).

#### **G. Protocol**

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 =  $0.8 \times 0.5 = 0.400$

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* IgA 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* IgA by ELISA.

0.9-1.1 Borderline positive. Follow-up testing is recommend if clinically indicated.

>1.1 Detectable antibody to *Chlamydia* IgA by ELISA.

**Limitations of the Procedure**

1. 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.
2. Lipemic or hemolyzed samples may cause erroneous results.

**References**

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