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# Chlamydia Trachomatis IgG ELISA Kit

**(Catalog # E4712-100; 96 assays; Storage at 4°C )**

## I. Introduction:

Chlamydia Trachomatis is one of the most common human pathogens. Of the 15 recognized serotypes, 4 (A, B, Ba, and C) have been shown to cause hyperendemic blinding trachoma, a disease which afflicts hundreds of millions of people in developing countries. Three serotypes (L-1, L-2, and L-3) are the causes of lymphogranuloma venereum (LGV), a sexually transmitted systemic disease. The other serotypes (D through K) have been associated with genital tract infections and sporadic cases of conjunctivitis in industrialized societies. High levels of anti-Chlamydia IgG antibody are of diagnostic value in chronic or systemic infections such as salpingitis, mechanical infertility, perihepatitis, epididymitis, Reiter's syndrome and pneumonitis.

BioVision's Chlamydia Trachomatis test employs the LGV type 2 broadly reacting antigen of Chlamydia Trachomatis. It will detect Chlamydia Trachomatis, Chlamydia Psittaci and Chlamydia Pneumoniae (TWAR) antibodies. Purified Chlamydia Trachomatis antigen is coated on the surface ofmicrowells. Diluted patient serum is added to wells, and the Chlamydia Trachomatis IgG specific antibody, if present, binds to the antigen. Allunbound materials are washed away. After adding enzyme conjugate, it binds to the antibody-antigen complex. Excess enzyme conjugate is washed off, and TMB Chromogenic Substrate is added. The enzyme conjugate catalytic reaction is stopped at a specific time. The intensity of the color generated is proportional to the amount of IgG specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

## II. Applications:

- This ELISA kit is used for the determination of specific IgG antibody to Chlamydia in human serum samples

## III. Sample Type:

- Serum

## IV. Kit Contents:

| Components | E4712-100 | Part Number |
|---|---|---|
| Microwell strips | 12 x 8 strips | E4712-100-1 |
| Sample Diluent | 22 ml | E4712-100-2 |
| Calibrator | 150 µl | E4712-100-3 |
| Negative Control | 150 µl | E4712-100-4 |
| Positive Control | 150 µl | E4712-100-5 |
| Wash Buffer (10X) | 100 ml | E4712-100-6 |
| Enzyme Conjugate | 12 ml | E4712-100-7 |
| TMB Chromogenic Substrate | 12 ml | E4712-100-8 |
| Stop Solution | 12 ml | E4712-100-9 |

## V. User Supplied Reagents and Equipment:

- Microplate reader capable of measuring absorbance at 450 nm
- Distilled or deionized water

## VI. Storage Conditions and Reagent Preparation:

The entire kit may be stored at 4°C

Keep microwells sealed in a dry bag with desiccants. Spin tubes briefly to bring down all components to the bottom of tubes. Reagents are stable until the expiration of the kit. Do not expose reagent to heat, sun, or strong light.

Bring all samples and kit reagents to room temperature (20-25 °C) and gently mix.

**Wash buffer:** Prepare 1X Wash buffer by adding distilled or deionized water to 10X wash buffer to a final volume of 1 liter

**Sample Preparation:** Collect blood specimens & separate the serum immediately. Samples may be stored refrigerated at (2-8°C) for 7 days. Store frozen at (-20°C) for up to six month. Avoid multiple freeze-thaw cycles. Prior to assay, frozen serum should be completely thawed and mixed well.

## VII. Assay Protocol:

1. Place the desired number of coated strips into the holder.
2. Prepare 1:40 dilutions by adding 5 µl of the **test samples, negative control, positive control, and calibrator** to 200 µl of **Sample Diluent.** Mix well.
3. Add 100 µl of **diluted serum, calibrator, and controls** into the appropriate wells. For blank, add 100 µl **Sample Diluent** in well. Tap to remove air bubbles from the liquid and mix well. Incubate for 30 minutes at room temperature.
4. Aspirate liquid from all wells. Repeat washing step three times with washing buffer.
5. Add 100 µl of **enzyme conjugate** to each well and incubate for 30 minutes at room temperature.
6. Remove enzyme conjugate from all wells. Repeat washing three times with washing buffer.
7. Add 100 µl of **TMB Chromogenic Substrate** to each well and incubate for 15 minutes at room temperature.
8. Add 100 µl of **Stop solution** to stop the reaction. **Note:** Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm using ELISA reader.

**VIII. Calculation:**

**To obtain Cut off OD value:** Multiply the OD of Calibrator by Factor (f) printed on label of Calibrator bottle. Calculate the Chlamydia IgA Index of each determination by dividing the OD values of each sample by obtained OD value of Cut off.

**Example Of Typical Results:**

If Factor ( f) value on label = 0.4
This factor (f) is a variable. It is specific for a lot manufactured and printed on label of Calibrator.
Obtained Calibrator O.D. = 1.100
Cut-off O.D. = 1.100 x 0.4 = 0.44 (By definition Chlamydia IgA Index = 1)
Patient sample O.D. = 0.580
Chlamydia IgA Index = 0.580 / 0.44 = 1.32 (Positive result)
Patient sample O.D. = 0.320
Chlamydia IgA Index = 0.320 / 0.44 = 0.73 (Negative result)

**Quality Control:**

The test run may be considered valid provided the following criteria are met:
1. The O.D. value of the blank should be less than 0.150.
2. If the O.D. value of the Calibrator is lower than 0.250, the test is not valid and must be repeated.
3. The IgA Index for Negative and Positive Control should be in the range stated on the CoA/labels.

**Interpretation:**

Negative: IgA Index of 0.90 or less is seronegative for IgA antibody.
Equivocal: IgA Index of 0.91 - 0.99 are equivocal. Sample should be retested.
Positive: IgA Index of 1.00 or greater.

**Limitation of the test:**

1. A single serum sample cannot be used to determine recent infection.
2. A serum specimen taken in an early stage during acute phase of infection may contain low levels of IgA antibody and render an IgA Index result negative.
3. As with other serological assays, the results of these assays should be used in conjunction with information available from clinical evaluation and other diagnostic procedures.

**Sensitivity and Specificity**

Sensitivity, specificity and accuracy were evaluated using a commercial available ELISA kit on 40 specimens. The correlation results are summarized in the following table:

| | | Reference ELISA | | |
|---|---|---|---|---|
| | | N | P | Total |
| MICROWELL ELISA | N | 69 (D) | 3 (B) | 72 |
| | P | 1 (C) | 31 (A) | 32 |
| | Total | 70 | 34 | 104 |

Sensitivity = A / (A+B)= 31 / (31+3) = 91.1%
Specificity = D / (C+D) = 69 / (69+1) = 98.5%
Accuracy (Overall agreement) = (A+D) / (A+B+C+D) = 100 / 104 = 96.1%

**Precision**:

The precision of the assay was evaluated by testing three different sera eight replicates on 3 days. The intra-assay and inter-assay C.V. are summarized below:

| N=8 | Negative | Low positive | Positive |
|---|---|---|---|
| Intra-assay | 10.9% | 10.5% | 8.9% |
| Inter-assay | 12.3% | 11.1% | 10.5 % |

**IX. Related Products:**

- Chlamydia Trachomatis IgG ELISA Kit (E4711)
- Mycoplasma DNA Kit (K1416)
- Mycoplasma Arginine Deiminase (ADI), Recombinant Protein (P1278)

***FOR RESEARCH USE ONLY! Not to be used on humans.***