DIAGNOSTIC AUTOMATION, INC.

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IVD	 See external label	 2°C-8°C	 Σ=96 tests	REF Cat # 1407Z

Chlamydia Trachomatis IgG

Cat #1407Z

Test	Chlamydia IgG ELISA
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	5µL Serum
Specificity	98.5%
Sensitivity	96.1%
Total Time	~ 90 min
Shelf Life	12 -18 Months

***** *Laboratory results can never be the only base of a medical report. The patient history and further tests have to be taken into account*

NAME AND INTENDED USE

The Diagnostic Automation ELISA, Chlamydia Trachomatis IgG is intended for use in evaluating a patient's serologic status to Chlamydia Trachomatis infection. It is also used to evaluate paired sera for the presence of a significant increase in specific IgG as indicative of a recent or current Chlamydia Trachomatis infection.

SUMMARY AND EXPLANATION OF THE TEST

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. These agents are the major recognized cause of nongonococcal urethritis in men, in whom they may also cause epididymitis. In women, C. trachomatis causes cervicitis and has been associated with acute salpingitis. Infants born through an infected birth canal may contract the infection and then develop inclusion conjunctivitis of the newborn and/or the characteristic chlamydial pneumonia syndrome.

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.

DIAGNOSTIC AUTOMATION ELISA 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.

PRINCIPLE OF THE TEST

Purified Chlamydia Trachomatis antigen is coated on the surface of microwells. Diluted patient serum is added to wells, and the Chlamydia Trachomatis IgG specific antibody, if present, binds to the antigen. All unbound 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.

MATERIAL PROVIDED

1. Microwell Strips: Chlamydia trachomatis antigen coated wells	(12 X 8 wells)
2. Sample Diluent: Blue color solution	1 Vial (22 mL)
3. Calibrator: Factor Value (f) stated on label. Red Cap	1 Vial (150 µL)
4. Negative Control: Range Stated on label. Naturaln Cap	1 Vial (150 µL)
5. Positive Control: Range Stated on label. Green Cap	1 Vial (150 µL)
6. Washing Concentrate 10X: White Cap	1 bottle (100 mL)
7. Enzyme Conjugate: Red color solution	1 Vial (12 mL)
8. TMB Chromogenic Substrate: Amber bottle	1 Vial (12 mL)
9. Stop Solution	1 Vial (12 mL)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 °C.
2. Always keep microwells tightly sealed in pouch with desiccants. We recommend you use up all wells within 4 weeks after initial opening of the pouch.
3. The reagents are stable until expiration of the kit.
4. Do not expose test reagents to heat, sun or strong light during storage or usage.

WARNINGS AND PRECAUTIONS

1. Potential biohazardous materials:
   The calibrator and controls contain human source components which have been tested and found nonreactive 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
2. Do not pipette by mouth. Do not smoke, eat, or drink in the areas in which specimens or kit reagents are handled.
3. The components in this kit are intended for use as a integral unit. The components of different lots should not be mixed.
4. This product contains components 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 of serum sample.

PREPARATION FOR ASSAY

1. Prepare 1x washing buffer. Prepare washing buffer by adding distilled or deionized water to 10x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 °C) and gently mix.

ASSAY PROCEDURE

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. 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 30 minutes at room temperature.
4. Remove liquid from all wells. Repeat washing three times with washing buffer.
5. Dispense 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. Dispense 100 µl of TMB Chromogenic Substrate to each well and incubate for 30 minutes at room temperature.
8. Add 100 µl of 2 **N** HCl to stop reaction.
   Make sure there are no air bubbles in each well before reading
9. Read O.D. at 450 nm with a microwell reader.

CALCULATION OF RESULTS

1. To obtain cut off OD Value: Multiply the OD of calibrator by factor (f) printed on label calibrator.
2. Calculate the Chlamydia IgG Index of each determination by dividing the OD value of each sample by obtained OD value of Cut-off.

For Example:
If Factor (f) value on label = 0.4
Obtained Calibrator O.D = 1.100
Cut off O.D = 1.100 X 0.4 = 0.44 (by definition Chlamydia IgG Index = 1)

Patient sample O.D = 0.580
Chlamydia IgG Index = 0.580/0.44 = 1.32 (Positive Result)

Patient Sample O.D = 0.320
Chlamydia IgG 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 reagent blank against air from a microwell reader should be less than 0.250.
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 IgG Index for Negative and Positive Control should be in the range stated on the labels.

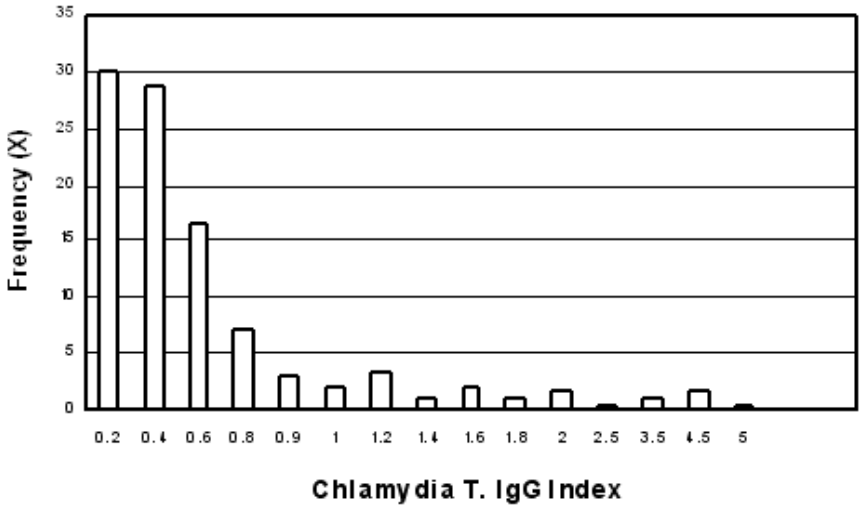
INTERPRETATION

Negative: IgG Index of 0.90 or less are seronegative for IgG antibody.

Equivocal: IgG Index of 0.91 - 0.99 are equivocal. Sample should be retested.

Positive: IgG Index of 1.00 or greater.

Expected Values: 230 random samples were determined with DIAGNOSTIC AUTOMATION microwell ELISA. Chlamydia Trachomatis IgG. The test results were computed as IgG Index using a chosen reference serum (cut off) as IgG index 1. The distribution of frequency versus IgG Index is presented as the following: 29 were found to be positive (12.6%), and 196 were found to be negative (85.2%).Histogram of Chlamydia T. IgG Index Total samples n=230



LIMITATIONS OF THE PROCEDURE

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 IgG antibody and render an IgG 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.

PERFORMANCE CHARACTERISTICS

Sensitivity and Specificity:
Sensitivity, specificity and accuracy were evaluated using a commercial available ELISA kit on 104 specimens. The correlation results are summarized in the following table:

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

Sensitivity = **A / (A+B) = 31 / (31+3) = 91.1%**

Specificity = **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%

Cross-reactivity:
A study was performed to determine the cross-reactivity of the test to the following antibodies:

1. IgG of EBV, Mumps, Measle, and VZV.
2. IgG and IgM of Rubella, Toxo, CMV, HSV 1, and HSV 2.
3. IgM of RF.
4. IgG of ANA, anti- ds DNA.

All positive samples tested give negative results.

REFERENCES

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SUMMARY OF ASSAY PROCEDURE

Step	(20-25°C Room temp.)	Volume	Incubation time
1	Sample dilution 1:40 = 5 µl / 200 µl		
2	Diluted samples, calibrator & controls	100 µl	30 minutes
3	Washing buffer (3 times)	350 µl	
4	Enzyme conjugate	100 µl	30 minutes
5	Washing buffer (3 times)	350 µl	
6	TMB Chromogenic Substrate	100 µl	30 minutes
7	Stop solution	100 µl	
8	Reading OD 450 nm		

Date Adopted	Reference No.
2010-10-28	DA-Chlamydia IgG-2010

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Revision Date: 11-03-2010