DIAGNOSTIC AUTOMATION, INC.

23961 Craftsman Road, Suite D/E/F, Calabasas, CA 91302
Tel: (818) 591-3030 Fax: (818) 591-8383
onestep@rapidtest.com
technicalsupport@rapidtest.com
www.rapidtest.com

See external label	2°C-8°C	Σ=96 tests	REF Cat # 1427Z

Chlamydia Trachomatis IgM

Cat# 1427Z

Test	Chlamydia IgM
Method	ELISA: Enzyme Linked Immunosorbent Assay
Principle	ELISA - Indirect; Antigen Coated Plate
Detection Range	Qualitative Positive; Negative control & Cut off
Sample	5ul Serum
Specificity	99%
Sensitivity	99%
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 IgM is intended for the determination of specific IgM antibody to Chlamydia in a single human serum sample, by an Enzyme-Linked Immunosorbent Assay.

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 IgM 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 IgM specific antibody in the sample. The results are read by a microwell reader compared in a parallel manner with calibrator and controls.

MATERIALS PROVIDED

1. Microwell Strips: Chlamydia trachomatis antigen coated wells	(12 X 8 wells)
2. Absorbent Solution: Black Cap	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. Natural 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 absorbent solution. Mix well.
3. Dispense 100 µl of diluted sera, calibrator, and controls into the appropriate wells. For the reagent blank, dispense 100 µl absorbent solution 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 IgM 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
This factor (f) is a variable value. It could be 0.35 or 0.5 etc. 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 IgM Index = 1)

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

Patient Sample O.D = 0.320
Chlamydia IgM 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 IgM Index for Negative and Positive Control should be in the range stated on the labels.

INTERPRETATION

Negative: IgM Index of 0.90 or less are serongative for IgM antibody.

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

Positive: IgM Index of 1.00 or greater.

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 IgM
   antibody and render an IgM 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

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	12.5%	10.2%	9.5%
Inter-assay	15.4%	12.5%	10.6%

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

1. IgM of EBV, Mumps, Measle, and VZV.
2. IgM of Rubella, Toxo, CMV, HSV 1, and HSV 2.
3. IgM of RF.

All positive samples tested give negative results.

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