ALTA CALIDAD EN PRUEBAS PARA SU LABORATORIO

Chemiluminescence ELISA

Chlamydia Trachomatis IgM

(96 tests)

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	Substrate A and Substrate B mixture	100 µl	5 minutes
7	Read with Luminometer in 5~30 minutes		

NAME AND INTENDED USE

The Chlamydia Trachomatis IgM Chemiluminescence ELISA is intended for the determination of specific IgM antibody to Chlamydia in a single human serum sample, by an Enzyme-Linked Immunosorbent Assay.

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.

Chlamydia Trachomatis IgM Chemiluminescence ELISA 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 substrate A & substrate B mixture is added. The light generated (RLU) is proportional to the amount of IgM specific antibody in the sample. The results are read by a microwell luminometer 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. 1 vial (22 ml)
3. Calibrator: Factor value (f) stated on label. 1 vial (150µl)
4. Negative Control: Range stated on label. 1 vial (150µl)
5. Positive Control: Range stated on label. 1 vial (150µl)
6. Washing Concentrate (H) 20x. 1 bottle (50 ml)
7. Enzyme Conjugate: Red color solution. 1 vial (12 ml)
8. Substrate A: H2O2 in buffer. 1 vial (6 ml)
9. Substrate B: Luminol in buffer. 1 vial (6 ml)

STORAGE AND STABILITY

1. Store the kit at 2 - 8 ^{o}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 ^{o}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 20x wash concentrate to a final volume of 1 liter.
2. Bring all specimens and kit reagents to room temperature (20-25 ^{o}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. Mix equal volume of Substrate A & Substrate B, then dispense 100 μl of this mixture to each well.
8. Read RLU with a microwell luminometer within 5~30 minutes.

CALCULATION OF RESULTS

1. To obtain Cut-off value: Multiply the RLU of Calibrator by Factor (f) printed on label of Calibrator.
2. Calculate the IgM Index of each determination by dividing the RLU values of each sample with obtained RLU value of Cut-off.

NOTE: This factor (f) is a variable.
It is specific for a lot manufactured and printed on label of Calibrator.

For example:

If Factor (f) value on label = 0.40

900110 x 0.40 = 360044

Sample	RLU	Mean RLU (A)	Calculated Cut off Value (B)	INDEX A/B	Interpretation
Calibrator f = 0.40	897142 903078	900110	360044		
Positive Control	1449370 1450960	1450165		4.02	Positive
Negative Control	6556 6978	6767		0.02	Negative
Patient Sample 1	1712547 1800691	1756619		4.88	Positive
Patient Sample 2	128620 118540	123580		0.34	Negative

QUALITY CONTROL

1. In order for the assay results to be considered valid, the controls should be within the ranges indicated on the labels.
2. The RLU values vary with the different luminometer used.
3. Each laboratory should assay controls at levels in low, normal and elevated ranges for monitoring assay performance. Quality control trends should be maintained to monitor batch to batch consistency.

INTERPRETATION

Negative: IgM Index of 0.90 or less are seronegative 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.

REFERENCES

1. Schachter, J. 1978. Chlamydial infections. N. Engl. J. Med. 298: 428-435, 490-495, 540-549.
2. Sarov, I., Kleinman, D., Cevenini, R., Holoberg, G., Potashnik, G. Sarov, B. and Insler, V. (1986). Specific IgG and IgA Antibodies to Chlamydia trachomatis in Infertile Women. Int. J. Fertil 31: 193-197.
3. Kaneti, J. et al. (1988). IgG and IgA Antibodies specific for Chlymydia trachomatis in Acute Epididymitis. Europ. Urol. 14: 323-327.
4. Kletter, Y., Caspi, D., Yarom, M., Sarov, B., Sarov, I. And Tanay. A. Serum IgA and IgG Antibodies Specific to Chlamydia in Patients with Rieter's Syndrome (RS). In: Proceedings of The European Society for Chlamydia Research, Societa Editrice Esculapio, Bologna. 1988. P. 170.
5. Paran, H., Heimer, D. and Sarov, I. (1986). Serological, Clinica and Radiological Findings in Adults with Bronchopulmonary Infections Caused by Chlamydia trachomatis. Isr. J. Med. Sci. 22: 823-827.