Chlamydia Trachomatis Antibody IgG GENLISA™ ELISA

REF : KBD10017

Ver 1.0

IVD

Enzyme Immunoassay for the Qualitative Determination of Chlamydia Trachomatis Antibody IgG in human serum and plasma.

IVD	**For In-vitro Diagnostic Use**	**REF**	**Catalog Number**
	**Store At**	**LOT**	**Batch Code**
	**Manufactured By**		**Biological Risk**
	**Expiry Date**		**Consult Operating Instructions**

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Introduction:

One of the most common sexually transmitted diseases (STDs) is urogenital Chlamydiosis (UC). According to WHO, each year about 130 million new cases of Chlamydia Trachomatis are registered worldwide, effecting about 8% of the population.
The causative agent of urogenital Chlamydiosis is Chlamydia Trachomatis, gram-negative intracellular parasites with a unique development cycle, which includes two forms of existence: metabolically active non-infectious intracellular reticular bodies and metabolically inactive extracellular infectious elementary bodies. Chlamydia Trachomatis can exit the host cell without lysis, which enables moderate infections.
The source of infection in UC is a person who has an acute or chronic form of the disease with a manifest or asymptomatic course of the process. Signs of Chlamydiosis are not very specific and practically do not differ from other STDs. The most common forms of the disease in men are urethritis and prostatitis, in most cases they have an asymptomatic course. In women, cervicilis and salpingitis are the most common manifestations of UC. Also, Chlamydial infection in women is the cause of serious reproductive disorders: chlamydiosis is detected in 40-70% of patients with tubal infertility. The presence of UC in a pregnant woman increases the risk of threatened miscarriage, premature birth, etc.
Diagnostics of chlamydiosis is quite difficult due to the nature of the pathogen life cycle. In recent years non-invasive methods for detecting Chlamydia infection have become increasingly popular. One method for detecting of Chlamydia Trachomatis DNA is polymerase chain reaction (PCR) and it is characterized by high sensitivity and specificity for detecting an active Chlamydial infection. However, the reliable PCR result largely depends of preparation and storage of the material. Serological methods (including ELISA) allow determining the pattern and stage of the disease, especially important in chronic Chlamydiosis which can last for many months and years. For this purpose, IgM, IgA and IgG specific antibodies are determined by ELISA.
Chlamydia Trachomatis-specific IgA antibodies are present in both serum and secretory forms and are indicators of both the acute infection and the manifestation of the disease in its chronic form. The serum IgA antibodies appear 10-14 days after the onset of the disease, slightly earlier than IgG antibodies but in low concentrations. They can be found early in the disease in genital secretions. High concentrations of antibodies of this class may indicate a chronic infection. Specific IgA antibodies have a half-life of 5-7 days, which allows them to be used to monitor the treatment effectiveness. A decrease in the level of these antibodies by 2-3 times indicates a successful therapy. IgG antibodies appear starting from the third week after the onset of the disease. Their presence reflects the overall picture of the immune response because of acute, chronic or past infection. In the latter case, IgG may be detected at a low level for many years.

Intended Use:

The Chlamydia Trachomatis Antibody IgG GENLISA™ ELISA kit is used as an analytical tool for Qualitative determination of Chlamydia Trachomatis Antibody IgG in serum, plasma and other biological samples.

Principle:

The method employs sandwich ELISA technique. Anti IgG antibodies are pre-coated onto microwells. Samples and standards are pipetted into microwells and Chlamydia Trachomatis Antibody IgG present in the sample are bound by the antibodies. Chlamydia Trachomatis conjugated to HRP is added and incubated to form a complex. After washing microwells in order to remove any non-specific binding, the substrate solution (TMB) is added to microwells and color develops proportionally to the amount of Chlamydia Trachomatis Antibody IgG in the sample. Color development is then stopped by addition of stop solution. Absorbance is measured at 450 nm.

Materials Provided:

1. Anti IgG Antibody Microtiter Coated Plate (12 x 8 wells) – 1 no
2. Positive Control – 0.5 ml/vial
3. Negative Control – 1.0 ml/vial
4. Sample diluent – 10 ml
5. Chlamydia Trachomatis: Conjugate Solution – 12 ml
6. (20X) Wash Buffer – 50 ml
7. TMB Substrate – 12 ml
8. Stop Solution – 12 ml
9. Instruction Manual

Materials to be provided by the End-User:

1. Microtiter Plate Reader able to measure absorbance at 450 nm.
2. Adjustable pipettes and multichannel pipette to measure volumes ranging from 25 ul to 1000 ul
3. Deionized (DI) water
4. Wash bottle or automated microplate washer
5. Graph paper or software for data analysis
6. Timer
7. Absorbent Paper

Handling/Storage:

1. Kit should be stored at 2-8°C upon receipt and when it is not in use.
2. Keep un-used wells in their sealed bag with desiccants.
3. Do not use expired date reagents.
4. Do not freeze.
5. Protect from light and moisture.

Health Hazard Warnings:

1. Reagents that contain preservatives may be harmful if ingested, inhaled or absorbed through the skin. Refer to the MSDS online for details.
2. To reduce the likelihood of blood-borne transmission of infectious agents, handle all serum and/or plasma in accordance with NCCLS regulations.

Specimen Collection and Handling:

Serum- Coagulate at room temperature for 10-20 minutes; centrifuge for 20-min at 2000-3000 rpm. Remove the supernatant. If precipitation appears, re-centrifuge.
Plasma- Use EDTA or citrate plasma as an anticoagulant, mix for 10-20 minutes; centrifuge for 15-min at 2000-3000 rpm. Remove the supernatant carefully. If precipitation appears, re-centrifuge.

Reagent Preparation:

1. Allow all components to reach RT (Room Temperature) prior to use in the assay.
2. Wash Buffer (20X) Dilution: To make Wash Buffer (1X), add 4ml concentrate Wash Buffer (20X) + 76 ml of DI water is sufficient for 8 wells. Once diluted, it is stable at 2-8°C for 7 days. This is the working solution.

Test Procedure:

1. All reagents should be allowed to reach room temperature before use.

2. Add **80 ul Sample Diluent** to respective blank wells.

3. Add **20 ul** of **Negative controls**, **Positive Control** and **Samples** to respective wells.

4. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

5. Aspirate and wash plate 5 times with diluted Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

6. Add **100 ul** of **diluted enzyme conjugate** to each well except blank well.

7. Cover the plate with a sealer and incubate for 30 minutes at 37°C.

8. Aspirate and wash plate 5 times with diluted Wash Buffer and blot residual buffer by firmly tapping plate upside down on absorbent paper. Wipe of any liquid from the bottom outside of the microtiter wells as any residue can interfere in the reading step.

9. Add **100 ul** of **TMB Substrate** into each well.

10. Do not cover the plate with a lid and incubate it in the dark for 15 minutes at 18-25°C.

11. Add 100 ul of Stop Solution to all wells. The wells should turn from blue to yellow in color.

12. Read the absorbance at 450 nm with a microplate within 10-15 minutes after addition of Stop solution

Validation of the test:

The test run may be considered valid provided the following criteria are met:

Positive Control	OD ≥ 1.200
Negative Control	OD ≤ 0.150

If one of the negative control absorbances does not match the above criteria, this value should be discarded and a mean value should be calculated using the other two values. If more than one negative control absorbance does not meet the criteria, the test is invalid and must be repeated.

Calculation of Results:

Calculate the mean absorbance value for 3 negative controls (Nc), Cut off value (CO) and $Ratio_{sample}$

Nc = (Nc1 + Nc2 + Nc3) / 3;

CO = Nc + 0.25;

$Ratio_{sample} = OD_{sample}/CO$,

OD_{sample} – optical density of the well containing sample.

Interpretation of Results:

Ratio $_{sample}$ >1.1	Positive
0.9 ≤ Ratio $_{sample}$ ≤ 1.1	Equivocal
Ratio $_{sample}$ < 0.9	Negative

If the result is equivocal, repeat the test. If it remains equivocal, collect a new serum sample.

Performance Characteristics:

Sensitivity
The relative sensitivity of Chlamydia Trachomatis Antibody IgG kit was calculated and determined to be **100%**

Specificity
The relative specificity of Chlamydia Trachomatis Antibody IgG kit was calculated and determined to be **100%**

Precision

Intra-Assay precision:
Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 32-replicate determinations using 1 lot of the test kit.

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Serum No	OD	Ratio	CV%
46S	2.127	7.56	3.1
39S	0.664	2.36	4.2

Inter-Assay precision:
Coefficient of variation (CV) was calculated by measuring 2 samples with various specific antibody levels in 4 ELISA performances during 4 days, in 8-replicate determinations.

Serum No	OD	Ratio	CV%
46S	2.059	7.25	4.2
39S	0.666	2.34	4.6

Safety Precautions:

- **This kit is For In-vitro Diagnostic Use only**. Follow the working instructions carefully.
- The expiration dates stated on the kit are to be observed. The same relates to the stability stated for reagents
- Do not use or mix reagents from different lots.
- Do not use reagents from other manufacturers.
- Avoid time shift during pipetting of reagents.
- All reagents should be kept in the original shipping container.
- Some of the reagents contain small amount of sodium azide (< 0.1 % w/w) as preservative. They must not be swallowed or allowed to come into contact with skin or mucosa.
- Source materials maybe derived from human body fluids or organs used in the preparation of this kit were tested and found negative for HBsAg and HIV as well as for HCV antibodies. However, no known test guarantees the absence of such viral agents. Therefore, handle all components and all patient samples as if potentially hazardous.
- Since the kit contains potentially hazardous materials, the following precautions should be observed
- Do not smoke, eat or drink while handling kit material
- Always use protective gloves
- Never pipette material by mouth
- Wipe up spills promptly, washing the affected surface thoroughly with a decontaminant.
- In any case GLP should be applied with all general and individual regulations to the use of this kit.

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