CE 0483

SeroELISA™ Chlamydia IgA

Enzyme-Linked Immunosorbent Assay (ELISA) for the detection of specific IgA antibodies to ***Chlamydia*** in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No. 113-01)

For In Vitro Diagnostic Use
For professional use only
Store at 2-8°C. **Do Not Freeze**

***Savyon*® Diagnostics Ltd.**
3 Habosem St. Ashdod 7761003
ISRAEL
Tel: +972.8.8562920
Fax: +972.8.8523176
E-mail: support@savyondiagnostics.com

Intended Use

The SeroELISA™ Chlamydia IgA kit is intended for the determination of specific IgA antibodies to Chlamydia in a single human serum sample by an Enzyme-Linked Immunosorbent Assay (ELISA).
For *In Vitro* Diagnostic Use.

Introduction

Chlamydia is a gram-negative obligate intracellular bacteria that causes acute and chronic diseases in mammalian and avian species. The genus Chlamydia is comprised of four species: *C.trachomatis*, *C.pneumoniae*, *C.psittaci* and *C. pecorum*.

C.trachomatis is divided into 15 serovars (1). Serovars A, B, Ba and C are agents of trachoma (2), the leading cause of preventable blindness endemic in third world countries. Serovars L_1-L_3 are the agents of lymphogranuloma venereum. Serovars D-K are the common cause of sexually transmitted genital infection worldwide: cervicitis, endometritis/ salpingitis (3) in females and urethritis (4) in both males and females. Endometritis/salpingitis can lead to tubal occlusion with a higher risk of extra uterine pregnancy and infertility. Genital infection may cause an acute and persistent infection occasionally without any clinical symptoms. Generally, these infections are treatable once they are diagnosed. However without any treatment the infection may progress to a severe chronic inflammation leading to infertility, ectopic pregnancy, induced abortion or premature delivery. Moreover, infants to infected mothers may be infected during birth, leading to conjunctivitis or pneumonia (5).

C.pneumoniae is an important respiratory pathogen in humans and causes up to 10% of community-acquired pneumonia cases. It has been associated with acute respiratory diseases, pneumonia, asthma, bronchitis, pharyngitis, acute chest syndrome of sickle cell disease, coronary heart disease, and Guillain-Barre syndrome (6).

C.psittaci infects a diverse range of host species from molluscs to birds to mammals and also causes severe pneumonia.

Serodiagnostic tests, which rely on specific immunologic markers, serve as a non-invasive diagnostic tool in identification of both distal and deep infections (7).
Chlamydia, specific IgA antibody appears to be a reliable immunological marker of primary, chronic and recurrent infections (8). Anti-Chlamydia IgA antibody is of diagnostic value in non-gonococcal urethritis (NGU) patients (9), in women with acute salpingitis and in women with mechanical infertility (10) in ectopic pregnancy, prostatitis, epididymitis, conjunctivitis, Reiter's syndrome (10) and pneumonitis.

SeroELISA™ Chlamydia test employs the L_2 serovar broadly reacting antigen of *C. trachomatis.* It will detect *C. trachomatis*, *C. psittaci* and *C. pneumoniae* (TWAR) antibodies.

Principle of Test

- Human serum to be tested is brought into contact with the antigenic material coating the microtiter wells. Specific antibody, if present in the patient serum will bind to the attached antigen, a complex is formed and all the serum components are washed away in the wash phase.
- Horseradish peroxidase (HRP) conjugated anti-human IgA (α chain specific) is added to the wells. If an antigen-antibody complex was formed in the previous step, the peroxidase conjugated antibody will bind to the antibody moiety of the complex. If no antigen-antibody complex was formed in the previous step, the conjugate is washed away in the wash phase.
- TMB-Substrate is added. A positive reaction is indicated by a blue to deep blue color which develops in the test wells following enzymatic reaction of the peroxidase moiety with peroxide and the chromogen reactant. After the enzymatic reaction is stopped by an acidic solution, the absorbance of the test wells is determined at 450nm by a spectrophotometer.
- The absorbance at 450nm is indicative of IgA anti-Chlamydia titer in patient serum specimens.

Assay Procedure

1. Chlamydia Antigen Attached to Solid Phase (Ag) + Serum positive for IgA Anti-Chlamydia (Ab_1)

↓

$AgAb_1$ Complex

2. $AgAb_1$ Complex + HRP Conjugated Anti-Human IgA (Ab_2)

↓

$AgAb_1Ab_2$ Complex

3. $AgAb_1Ab_2$ Complex + TMB-Substrate

↓

Blue Solution

↓ ← Chromogen Stop Solution

Yellow Solution

(Absorbance Determination at 450nm)

Warning and Precautions

- **Warning:** THE CHLAMYDIAL ANTIGENIC MATERIAL HAS BEEN INACTIVATED AND CONTAINS NO DETECTABLE LIVE ORGANISMS. HOWEVER, THE STRIPS SHOULD BE HANDLED AND DISPOSED OF AS WOULD ANY POTENTIALLY BIO-HAZARDOUS LABORATORY MATERIAL.
  Precautions: This kit contains human sera which have been tested by FDA approved techniques, and found to be negative for HBV antigen and for HCV and HIV 1 and HIV 2 antibodies. Since no known method can offer complete assurance that products derived from human blood do not transmit infection, all human blood components supplied in this kit must be handled as potentially infectious serum or blood according to the recommendations published in the CDC/NIH manual "Biosafety in Micro Biological and Biomedical Laboratories, 1988".
- Substrate/Chromogen Solution is an irritant material to skin and mucous membranes. Avoid direct contact.
- **For *In-vitro* Diagnostic Use**.

Kit Contents

1. Precoated microtiter plate (96 wells per frame). Each sachet contains one microtiter plate comprising 12 removable strips in a plastic frame. Each strip is coated with Chlamydia antigens.
   1 Unit
2. Positive Control (human serum positive for anti-Chlamydia IgA antibody). Ready-to-Use.
   1 Vial, 2.0ml
3. Low Positive Control (human serum low positive for anti-Chlamydia IgA antibody). Ready-to-Use.
   1 Vial, 2.0ml
4. Negative Control (human serum negative for anti-Chlamydia IgA antibody). Ready-to-Use.
   1 Vial, 2.0ml
5. Ready To Use HRP Conjugated Anti-Human IgA (α-chain specific).
   1 Vial, 10ml
6. Serum Diluent, Ready-to-Use.
   2 Bottle, 60ml
7. Concentrated Wash Buffer (x20).
   1 Bottle, 100ml
8. TMB Substrate. Ready-to-Use.
   1 Vial, 16ml
9. Stop Solution (1M H_2S0_4), Ready-to-Use.
   1 Bottle, 16ml
10. Plate cover. **1 Unit**
11. Instruction Manual. **1**

Materials Required But Not Supplied

1. Perfectly clean test tubes for dilution of patient's sera.
2. Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
3. Disposable plastic pipettes (assorted sizes) and safety pipetting devices.
4. One liter volumetric flask.
5. One 50ml volumetric cylinder.
6. ELISA plate washer or wash bottle.
7. Paper towels or absorbent paper.
8. Vortex mixer.
9. A 37°C water bath with a lid, or a moisture chamber placed in a 37° ± 1°C incubator.
10. ELISA-reader with 450nm filter.
11. Distilled or double deionized water for the dilution of Concentrated Wash Buffer.

Storage and Shelf Life of Reagents

All the materials supplied should be stored at 2° to 8°C. If kept at 2° to 8°C the test reagents are stable until the expiry date indicated on the kit pack. Exposure of originally stoppered or sealed components to ambient temperature for a few hours will not cause damage to the reagents. **DO NOT FREEZE!**

When a kit is in use, the shelf life of the original material is 60 days from the day first opened. Once opened, the aluminum foil sachet containing the strips should be sealed with a tape. The dehydrating packet should not be removed.

Crystals may form in the 20x concentrated Wash Buffer during cold storage, this is perfectly normal. Redissolve the crystals by warming the buffer to 37°C before diluting. Once diluted, the solution may be stored at 2-8°C up to twenty-one days.

Specimen Collection

Serum specimens should be collected aseptically and stored at 2° to 8°C with 0.05% sodium azide (NaN_3) as a preservative if they are to be tested

within a few days. For longer periods, aliquots of serum specimens should be stored at -20°C.
Since turbid or hemolytic serum samples may give less reproducible results, it is highly recommended that serum samples tested be clear and non hemolytic.

Assay Procedure

Notes:

a) The components of this kit have been tested as one unit. Do not mix components from different kit lots or other manufacturer's kits.
b) All reagents should reach room temperature before use. Serum Diluent and Conjugate Diluent gelatinize when refrigerated. If needed, accelerate liquefaction by warming these components at 37°C for several minutes.
Salt crystals may form in the Concentrated Wash Buffer when stored at 2° to 8°C. These crystals should be completely redissolved by warming at 37°C before dilution.
c) Do not perform the test in the presence of reactive vapors (e.g. from acid, alkaline or aldehyde substances) or dust, since the enzymatic activity of the HRP conjugated Anti-Human IgA may be affected.
d) Do not touch the top of the strips. Do not touch the edges of the wells with the pipette tips when dispensing reagents.
e) Use disposable pipette tips. Avoid cross contamination between reagents.
f) Tap vial lightly on hard surface to free liquid that might be entrapped in the cap.
g) Avoid entrapping air bubbles in the wells.
h) Dispense liquids slowly to avoid spraying.
i) Positive Control, Low Positive Control and Negative Control sera should be run together with serum specimens each time the test is performed.
j) One well per test should be used for a blank value each time the test is performed.
k) All the procedure steps should be performed sequentially without interruption.

Test Procedure –Manual

Automation protocol available upon request

A) Pre-Washing of Strips

Prewashing of strips before starting the assay is advisable to decrease background noise signal.

1. Remove required number of strips from their aluminum foil sachets and insert them in the plate frame.
2. Dilute the Concentrated Wash buffer 1:20 with distilled water. For 100ml of Wash Buffer use 5ml of Concentrated Wash Buffer with 95ml distilled water and mix well.
Wash Buffer working solution should be prepared before use and excess discarded.
3. Wash the strips with the washing buffer, then discard the strip contents. Repeat this step **one** more time.
4. Dry the top of the strips and frame by gently tapping them over clean absorbent paper.

B) Incubation of Serum Samples and Controls

5. Dilute each **patient serum** 1:64 with Serum Diluent as follows:
1:16 -Add 10µl of patient serum to 150µl of Serum Diluent.
1:64 -Add 30µl of 1:16 dilution to 90µl of Serum Diluent.
Controls are provided in a ready-to-use form and should not be diluted.
6. Pipette 50µl of ready-to-use Positive control, Low Positive Control, Negative Control and 1:64 patient serum dilution into corresponding separate wells of the test strips.
Pipette 50µl of Serum Diluent into one well for blank value.
Pipetting of controls and serum specimens into the wells should not exceed 10 minutes.
7 Cover the strips with a plate cover and incubate for 30 minutes at 37ºC in a moisture chamber.
8. Discard the liquid content of the wells. Wash the wells **five** times and dry as in steps A).

C) Incubation with Conjugate

9. Pipette 50µl of ready to use HRP Conjugated Anti-Human IgA into each well.
10. Cover the strips with a plate cover and incubate for 30 minutes at 37ºC in a moisture chamber.
11. Discard the liquid content of the wells, wash them **five** times and dry as in steps A).

D) Incubation with TMB-Substrate

12. Pipette 100µl of TMB-Substrate into each well.
13. Cover the strips with a plate cover and incubate at room temperature for 30 minutes.
14. Stop the reaction by adding 100µl Stop Solution to each well.
Pipette the Stop Solution in the same sequence and at the same time intervals as the TMB Substrate.
15. Calibrate the spectrophotometer on the blank well. Determine the absorbance at 450nm and record the results.
Immediate determination of the absorbance is advisable but not mandatory. Absorbance determination should not exceed 30 minutes, following stopping of chromogenic reaction.

Acceptability of Criteria of the Test

A test-run is valid if:

- Positive Control absorbance is ≥0.8 at 450nm
- Low Positive Control absorbance is ≥0.4 at 450nm
- Negative Control absorbance is ≤0.15 at 450nm

If these conditions are not fulfilled, the test run is invalid and should be repeated.

Calculation of Cut-Off Value (COV)

The cut-off value is calculated according to the following formula:

$$COV = 0.198 \times (Pc - Nc) + Nc$$

Pc = Absorbance of Positive Control at 450nm
Nc = Absorbance of Negative Control at 450nm

Interpretation of Test Results

Absorbance at 450nm	Interpretation of Results	Estimated Chlamydia IgA Titer
Below COV – 0.03	Negative	<64
COV ± 0.03	Equivocal*	64
Above COV +0.03 up to Low Positive Control	Low Positive	64-128
Above Low Positive control up to Positive Control	Positive	256-512
Above Positive Control	High Positive	≥512

* Retest serum samples classified equivocal. If equivocal result is repeated testing of subsequent serum sample is recommended.

Significance of Results

Clinical experience indicates that IgA antibodies to Chlamydia may serve as a marker for active or chronic Chlamydia infection (3,11,12).

Endpoint Titer Determination

For monitoring of the immune profile of a patient, acute stage and convalescent serum samples should be compared. Endpoint titration of paired sera should be performed with both serum samples tested in the same run. The highest serum dilution with absorbance above the test cut-off value is the **end titer**.

Limitations of Assay

- No single serological test should be used as the only criterion for diagnosis. All clinical and laboratory data should be taken into account.
- The test is a single serovar (L_2) ELISA. L_2 contains antigenic determinants existing in serovars of *Chlamydia trachomatis* as well as the group antigen. Antibodies against *Chlamydia psittaci*, *Chlamydia pneumoniae* (TWAR) and *Acinetobacter calcoaceticus* may be detected by this ELISA.
- The significance of specimen titers must be considered in relation to the characteristics of the population being tested. These characteristics would include age, geographical location and sexual behavior, among other factors.
- This test will not indicate the site of chlamydia infection(s). It is not intended to replace cell culture isolation, if available.
- The absence of measurable serum antibodies does not exclude the possibility of a chlamydial infection.
- Bacterially contaminated or hyperlipaemic serum may cause erroneous results.

Performance Characteristics

SeroELISA™ Chlamydia test was compared to IPAzyme™ Chlamydia IgG/IgA test (Savyon Diagnostics product, Cat. No. 011-01) which is an accepted serological test for detection of IgA antibody to Chlamydia.
The study population included patients with suspected Chlamydia infections as well as healthy individuals (n=200).

Comparison of SeroELISA™ with IPAzyme™

SeroELISA™ / IPAzyme™	Positive	Negative	Total
Positive	96	4	100
Negative	7	93	100
Total	103	97	200

Overall agreement: (189/200) x 100 = 94.5%

Cross Reaction

Hospitalized patients, infected with *Neisseria gonorrhea*, *Staphylococcus aureus* and *Peptostreptococcus anaerobius*, who were diagnosed by commercial serology kits, were also tested with the SeroELISA Chlamydia kit. There was no significant cross-reaction detected.

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Glossary of Symbols:

Symbols for IVD components and Reagents			
	Manufacturer	IVD	In vitro diagnostic medical device
EU REP	EU Authorized representative		Consult instructions for use
Σ n	Contains Sufficient for <n> tests		Temperature limitation
REF	Catalogue Code		Use by Date
LOT	Batch Number	CE	CE mark
	Danger		Warning

CE 0483

EU REP

European Authorized Representative:
MedNet EC-REP GmbH
Borkstrasse 10,
48163 Muenster, Germany