savyonDIAGNOSTICS

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SeroCT™ IgG

REF A181-01M
REF B181-01M

ELISA for the detection
of IgG antibodies to
Chlamydia trachomatis
in human serum

For professional use only

CE 0483

Intended Use

The SeroCT™ - IgG kit is intended for the detection of IgG antibodies specific to *C. trachomatis* in human serum.
The Savyon® SeroCT™ - IgG kit is a new generation qualitative ELISA test which is based on *Chlamydia trachomatis* specific synthetic peptides.
SeroCT™ is used as an aid in the diagnosis of *C. trachomatis* specific infection.
SeroCT™ - IgG is intended to be run and interpreted in conjunction with the Savyon® SeroCT™ - IgA kit.

For ***In Vitro*** Diagnostic Use.

Introduction

Chlamydia is a gram negative obligate intracellular bacteria that causes acute and chronic disease in mammalian and avian species. The genus Chlamydia is comprised of four species: *C. trachomatis, C. pneumoniae,C. psittaci and C. pecorum* (1- 4).

C. trachomatis is divided into 15 serovars (5-8). Serovars A, B, Ba and C are agents of trachoma(9), 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(10) in females and urethritis(11) in both males and females. Endometritis/salpingitis can lead to tubal occlusion with a higher risk of extrauterine pregnancy and infertility. Genital infection may cause an acute and persistent infection occasionally without any clinical signs. 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 child delivery. Moreover, infants to infected mothers can be infected during birth leading to conjunctivitis or pneumonia (12-14).
The serology of *C. trachomatis* is more interesting in cases of chronic infections than in acute infections.

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 Gullain-Barré syndrome (15-17).

C. psittaci infects a diverse range of host species from molluscs to birds to mammals and also causes severe pneumonia. In animals, *C. psittaci* and *C. pecorum* are capable of inducing diverse disease syndromes, like pneumonia, enteritis, polyserositis, encephalitis and conjunctivitis.

Serological testing, now an established approach in many countries, has been shown to provide a comprehensive answer for the detection of C. *trachomatis* infection. In suspected deep-seated infections, serum sampling reduces the necessity for invasive procedures which are required for direct antigen detection. In cases of lower urogenital infections, collection limitations such as effectiveness of scrape sampling procedure,

specimen handling and transportation difficulties have to be weighted. Above all, there remains the issue that most Chlamydial infections are asymptomatic. Therefore, an infection may persist for a long time, ascend the upper genital tract causing deep and chronic infection, and increase the probability of false negative results in direct antigen detection.
Serological testing for *C. trachomatis*, through the detection of various specific antibodies, is today an effective and highly accepted method option(10,11,18,19). New and accurate technologies apply the immuno markers IgM, IgA and IgG to characterize the presence and stage of the infection.

Specific IgM is indicative of acute Chlamydial infection. Absence does not, however, preclude the presence of on-going infection, especially in recurrent and chronic cases. The use of specific IgA as a marker for active Chlamydial infection has been shown to have an important role because of its short half life time, while persisting as long as antigenic stimulation exists. IgA, however, is more suitable for post therapy follow-up. IgG is a marker for Chlamydial positive immune-response in either current, chronic or past infections.

Serological cross reactions occur between the three different species of Chlamydia. Most of the serological diagnostic assays for Chlamydia use either purified elementary bodies: microimmunofluorecence (MIF) and ELISA tests, lipopolysaccharide (LPS), or purified major outer membrane protein, (MOMP) as antigens. Genus specific epitopes are present in all the above antigens, therefore, low species specificity is observed. Moreover, a large proportion of the population has been exposed to *C. pneumoniae* (with no clinical signs), the prevalence of anti-Chlamydia antibodies is very high. Therefore, the differentiation between *C. pneumoniae* and *C. trachomatis* specific antibodies using conventional serological screening tests (MIF, ELISA, EIA etc.) is insufficient.

Savyon® Diagnostics has developed an assay in which *C. trachomatis* species specific epitopes, derived from different serotypes, are used in an Enzyme - Linked Immunosorbent Assay (ELISA). The test excludes cross-species reactive epitopes and enables more accurate and more specific determination of *C. trachomatis* IgG and IgA antibodies.

Principle of the Test

- SeroCT™ plates are coated with *C. trachomatis* specific peptides.

- Serum to be tested is diluted and incubated with the precoated SeroCT™ plate 1h at 37°C. In this step *C. trachomatis* specific antibodies are bound to the immobilized *C. trachomatis* specific peptides.

- Non specific antibodies are removed by washing.

- Anti-human IgG conjugated to horseradish peroxidase (HRP) is added and incubated 1h at 37°C. In this step the HRP-conjugate is bound to the prebound antigen-antibody complex.

- Unbound conjugate is removed by washing.
- Upon the addition of TMB-substrate, the substrate is hydrolyzed by the peroxidase, yielding a blue solution of the reduced substrate.
- Upon the addition of the stop solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450nm.
- The absorbance is proportional to the amount of the specific antibodies which are bound to the immobilized peptides.

Summary of Procedure

Wells of microtiter plate coated with *C. trachomatis* specific antigens

↓

Add 2 x 50µl of Negative Control
Add 1 x 50µl of Positive Control and diluted specimens

↓

Cover plate and incubate 1h at 37°C at 100% humidity

↓

Wash 3 times with Wash Buffer

↓

Add 50µl of 1/300 diluted HRP - Conjugate

↓

Cover plate and incubate 1h at 37°C at 100% humidity

↓

Wash 3 times with Wash Buffer

↓

Add 100µl of TMB-Substrate

↓

Cover plate and incubate 15min at room temperature

↓

Add 100µl of Stop Solution

↓

Read absorbance at 450nm

↓

Calculate and interpret results

Kit contents:

Test Kit for 96 determinations — Catalog No.: A181-01M

1. ***C. trachomatis*** **antigen - coated microtiter plate** 96 break-apart wells (8x12) coated with *C. trachomatis* specific peptides, packed in an aluminum pouch containing a desiccant card.

   1 plate

2. **Concentrated Wash Buffer (20x):** A PBS -Tween buffer.

   1 bottle, 100 ml

3. **Serum Diluent (Blue):** A ready to use buffer solution. Contains less than 0.05% proclin as preservative.

   1 bottle, 30 ml

4. **Conjugate Diluent (Green):** A ready to use buffer solution. Contains less than 0.05% proclin as preservative.

   1 bottle, 40 ml

5. **Negative Control:** A ready to use *C. trachomatis* IgG negative human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.

   1 vial, 2.5 ml

6. **Positive Control:** A ready to use *C. trachomatis* IgG positive human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.

   1 vial, 2.0 ml

7. **Concentrated HRP-Conjugate (300x):** Horseradish peroxidase (HRP) conjugated anti-human IgG (Gamma chain specific). Contains less than 0.05% proclin as preservative.

   1 vial, 0.2 ml

8. **TMB-Substrate:** A ready to use solution contains 3,3'5,5' tetramethylbenzidine as a chromogen and peroxide as a substrate.

   1 bottle, 14 ml

9. **Stop Solution:** A ready to use solution. Contains 1M H_2SO_4.

   1 bottle, 15 ml

10. **Plate cover:** **1 unit**
11. **Instruction Manual:** **1**

Test Kit for 192 determinations — Catalog No.: B181-01M

1. ***C. trachomatis*** **antigen - coated microtiter plate** 96 break-apart wells (8x12) coated with *C. trachomatis* specific peptides, packed in an aluminum pouch containing a desiccant card.

   2 plates

2. **Concentrated Wash Buffer (20x):** A PBS -Tween buffer.

   2 bottles, 100 ml each

3. **Serum Diluent (Blue):** A ready to use buffer solution. Contains less than 0.05% proclin as preservative.

   1 bottle, 60 ml

4. **Conjugate Diluent (Green):** A ready to use buffer solution. Contains less than 0.05% proclin as preservative.
1 bottle, 80 ml
5. **Negative Control:** A ready to use *C. trachomatis* IgG negative human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
1 vial, 2.4 ml
6. **Positive Control:** A ready to use *C. trachomatis* IgG positive human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
1 vial, 1.25 ml
7. **Concentrated HRP-Conjugate (300x):** Horseradish peroxidase (HRP) conjugated anti-human IgG (Gamma chain specific). Contains less than 0.05% proclin, as preservative.
1 vial, 0.2 ml
8. **TMB-Substrate:** A ready to use solution contains 3,3'5,5' tetramethylbenzidine as a chromogen and peroxide as a substrate.
1 bottle, 24 ml
9. **Stop Solution:** A ready to use solution. Contains 1M H_2SO_4.
1 bottle, 30 ml
10. **Plate cover:** **2 units**

11. **Instruction Manual:** **1**

Materials Required But Not Supplied:

1. Clean test tubes for dilution of patients sera.
2. Disposable plastic vial for dilution of the concentrated HRP- conjugated anti human IgG.
3. Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
4. One liter volumetric flask.
5. One 50ml volumetric cylinder.
6. Wash bottle.
7. Absorbent paper.
8. Vortex mixer.
9. A 37°C water bath with a lid, or a moisture chamber placed in an incubator at 37°C.
10. ELISA-reader with 450nm filter.
11. Distilled or double deionized water.

Warning and Precautions

For *In Vitro* Diagnostic Use

1. This kit contains human sera which have been tested by FDA and CE approved techniques, and found to be negative for HBV antigen and for HCV and HIV 1&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.
2. TMB-Substrate solution is an irritant material to skin and mucous membranes. Avoid direct contact.
3. Diluted Sulfuric acid (1M) is an irritant agent for the eyes and skin. In case of contact with eyes, immediatley flush area with water and consult a physician. Do not pour water into this product. In case of an accident or discomfort consult a physician (if possible present the label).
4. All the components of this kit have been calibrated and tested by lot.
   It is not recommended to mix components from different lots since it might affect the results.

Storage and Shelf-Life of Reagents

1. All the reagents supplied should be stored at 2-8°C. The unopened reagent vials are stable until the expiration 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 !**
2. Once the kit is opened, it's shelf life is 90 days.
3. Unused strips must be resealed in the aluminum pouch with the desiccant card, by rolling the open end and sealing tightly with tape over the entire length of the opening.
4. 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 for up to 21 days.

Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbidor contaminated sera is not recommended. Particulate material and precipitates in sera may cause erroneous results. Such specimens should be clarified by centrifugation or filtration prior to the test.

Storage:

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If a longer storage period is anticipated, aliquot and store the specimens below -20°C. Avoid repeated thawing and freezing.

Test Procedure - Manual

Automation protocol available upon request

A. Preparation of Reagents

1. Bring all components and clinical specimens to be tested to room temperature. Mix well the Positive Control, Negative Control and the clinical specimens before use.

2. Determine the total number of specimens to be tested. In addition to the specimens, the following must be included in each test: two wells of Negative Control and one well of Positive Control.

3. Withdraw the microtiter plate from its aluminum pouch by cutting one end near the seal. Leave the required number of strips (according to the number of specimens to be tested) in the 96 well frame.

4. Dilute the Concentrated Wash Buffer 1/20 with double-deionized or distilled water. For example, in order to prepare one liter of Wash Buffer, add 50ml of the Concentrated Wash Buffer to 950ml of double-deionized or distilled water.

B. Incubation of sera samples and controls

5. Dilute each patient serum 1/21 with the supplied Serum Diluent as follows: Add 10µl of patient serum to 200µl of Serum Diluent.

6. Pipette 50µl of Positive Control, Negative Control and 1/21 diluted sera into separate wells of the test strip.
   The Negative Control should be pipetted into two separate wells.

7. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
8. Discard the liquid content of the wells.

9. **Washing step:** Fill each well with wash buffer (300-350µl) up to the end of the well and discard the liquid, repeat this step two times, for a total of three washing steps.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with Conjugate

11. Concentrated HRP-Conjugate anti-human IgG should be diluted to working solution shortly before use.
    Dilute the concentrated HRP-conjugated 1/300 with Conjugate Diluent.
    For example, for two strips prepare a minimum of 3 ml of diluted HRP-Conjugate- (10µl of Concentrated HRP-conjugate is mixed with 3ml of Conjugate Diluent).

12. Pipette 50µl of diluted conjugate into each well .

13. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.

14. Discard the liquid content and wash as described in steps 9-10.

D. Incubation with TMB - Substrate

15. Pipette 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes.**

16. Stop the reaction by adding 100µl of Stop Solution (1M H_2SO_4) to each well.

E. Determination of Results

17. Determine the absorbance at 450nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

Note: *Any air bubbles should be removed before reading.*
The bottom of the ELISA plate should be carefully wiped.

Test Validation

For the test to be valid the following criteria must be met. If these criteria are not met the test should be considered invalid and should be repeated.

1. **Positive Control:** The absorbance value should be ≥ 0.8 at 450nm.

2. **Negative Control:** The average absorbance value of the Negative Control performed in duplicate should be 0.1< NC ≤ 0.4 at 450nm.

Calculation of Cut-Off Value (COV) and Cut-Off Index (COI)

The cut-off value is calculated according to the following formula: **COV = NC x 2**

NC = The average absorbance at 450nm of the Negative Control run in duplicate.

In order to normalize the results obtained in different tests, the cut-off index (COI) is calculated according to the following formula:

$$\text{COI} = \frac{\text{absorbance of the serum sample at 450nm}}{\text{COV}}$$

Interpretation of Results

Table 1: Correlation between the absorbance at 450nm and the presence of *C. trachomatis* IgG Antibodies

Absorbance at 450nm O.D	COI	Result	Interpretation of Results
O.D < COV	< 1.0	Negative	No detectable IgG antibodies to C. *trachomatis.*
COV≤ **O.D** ≤ COV x 1.1	1-1.1	Borderline	Presence or absence of detectable (Borderline) levels of IgG antibodies to C. *trachomatis* cannot be determined. A second serum sample should be obtained after 14-21 days and tested. (When second sample is borderline the result should be considered as negative).
O.D >COV x 1.1	>1.1	Positive	Detectable levels of IgG antibodies to *C. trachomatis.*

Table 2: Interpretation of results based on IgG and IgA antibodies determination

Levels of *C. trachomatis* specific antibodies		Interpretation of Results
IgG	**IgA**	
Negative	Negative	Negative (or beyond the sensitivity of this test).
Positive	Negative or Borderline	May indicate past or current infection.
Borderline	Borderline	Second sample testing is required after 14-21 days. Repeated borderline results should be considered negative.
Positive	Positive	May indicate acute or chronic infection.
Negative	Positive	May indicate acute or chronic infection.

Test Limitations

1. No single serological test should be used for a final diagnosis. All clinical and laboratory data should be taken into account.
2. Samples obtained too early during primary infection may not contain detectable antibodies. If Chlamydial infection is suspected, a second sample should be obtained 14-21 days later and tested in parallel with the original sample.

Performance Characteristics of SeroCT™-IgG

Table 3: Sensitivity of SeroCT™-IgG compared to culture.

The study was carried out in a reference laboratory on patients with a positive *C. trachomatis* culture.

Culture Positive	SeroCT™-IgG	
	Positive	**Negative**
45	35	10

Sensitivity: 35/45 x 100 = 78%

Table 4: Sensitivity and Specificity of SeroCT™ - IgG compared to microimmunofluorescence (MIF)

The study was carried out on patients suspected of having a *C. trachomatis* infection. SeroCT™ - IgG was compared to a commercial MIF test kit.

MIF		SeroCT™ - IgG	
		Positive	**Negative**
Positive	58	55	3
Negative	50	5	45
Total	108	60	48

Sensitivity: 55/58 x 100 = 95%
Specificity: 45/50 x 100 = 90%
Overall Agreement: 100/108 x 100 = 93%

Table 5: Specificity of SeroCT™-IgG on different control groups

Group Tested	No. of Sera	Negative on SeroCT™ - IgG	Specificity of SeroCT™ - IgG(%)
Blood Donors	250	230	92
Individuals Negative fo *C. trachomatis* and Positive for *C. pneumoniae* (MIF)	35	33	94
Healthy Children	30	29	97
Healthy Pregnant Women	30	28	93

Table 6: Specificity of SeroCT™-IgG compared to two different MIF tests

Specificity of SeroCT™-IgG was determined in comparison to MIF in two independent studies, each using a different MIF test. The serum samples used in each study were defined by MIF as either negative for both *C. trachomatis* and *C. pneumoniae* antibodies (MIF Ct-/Cp-) or as negative for *C. trachomatis*, positive for *C. pneumoniae* (MIF Ct-/Cp+).

	MIF Ct-/Cp-	MIF Ct-/Cp+	SeroCT™-IgG Negative	Specificity of SeroCT™-IgG(%)
Study #1 (In house MIF)	0	64	58	91
Study #2 (SeroFIA™ Savyon)	30	100	117	90

Conclusion: SeroCT™-IgG demonstrates a specificity higher than 90% for *C. trachomatis* .

Precision

Intra-assay (within-run) precision of the SeroCT™ - IgG test is shown below:

Sample	No. of Replicates	Mean Value	CV %
Positive	10	0.835	2.5
Negative	10	0.149	8.8

Inter-assay (between-run) precision of the SeroCT™-IgG is shown below:

Sample	No. of Replicates	Mean Value	CV %
Positive	10	0.902	2.9
Negative	10	0.167	5.5

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