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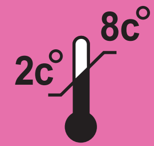
SeroCP™ IgG

REF A191-01M
REF B191-01M

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

IVD



For professional use only

CE 0483

SeroCP™ IgG

Intended Use

SeroCP™ IgG kit is intended for the detection of IgG antibodies specific to *Chlamydia pneumoniae* in human serum.
The SeroCP™ IgG kit is a qualitative Enzyme Linked Immunosorbent assay (ELISA), which is used as an aid in the diagnosis of *Chlamydia pneumoniae* infection.

For **In Vitro** Diagnostic Use.

Introduction

Chlamydia pneumoniae (TWAR) is an emerging infectious agent with a spectrum of clinical manifestations, including upper and lower respiratory tract infections (1). The majority of C. pneumoniae infections are mild and asymptomatic yet, may cause serious diseases, such as pharyngtitis, sinusitis, acute bronchitis and community acquired pneumonia. Undetected and untreated infection may lead to prolonged and persistent disease. Recent data indicates a possible association between *C. pneumoniae* infection and chronic diseases (2).
Seroprevalence of *C. pneumoniae* among children is low, increases sharply until middle age, and remains high in old age (>50%).

Difficulties in sample collection and inaccessibility of the infected site seriously affect the usefulness of direct detection methods. Therefore, serological testing is routinely used and serves as a non-invasive tool in identification of both distal and chronic chlamydial infections (3), where direct detection methods are rarely efficient (4). In addition, the presence of certain antibody types may also indicate the state of the disease.
Primary chlamydial infection is characterized by a predominant IgM response within 2 to 4 weeks and a delayed IgG and IgA response within 6 to 8 weeks. After acute *C. pneumoniae* infection, IgM antibodies are usually lost within 2 to 6 months (5), IgG antibody titers usually decrease slowly; whereas IgA antibodies tend to disappear rapidly (6). When primary chlamydial infection is suspected, the detection of IgM is highly diagnostic (7). However, in recurrent or chronic infections the prevalence of IgM is low and therefore absence of IgM does not necessarily exclude on-going infection.
In reinfection, IgG and IgA levels rise quickly, often in one to two weeks (8).

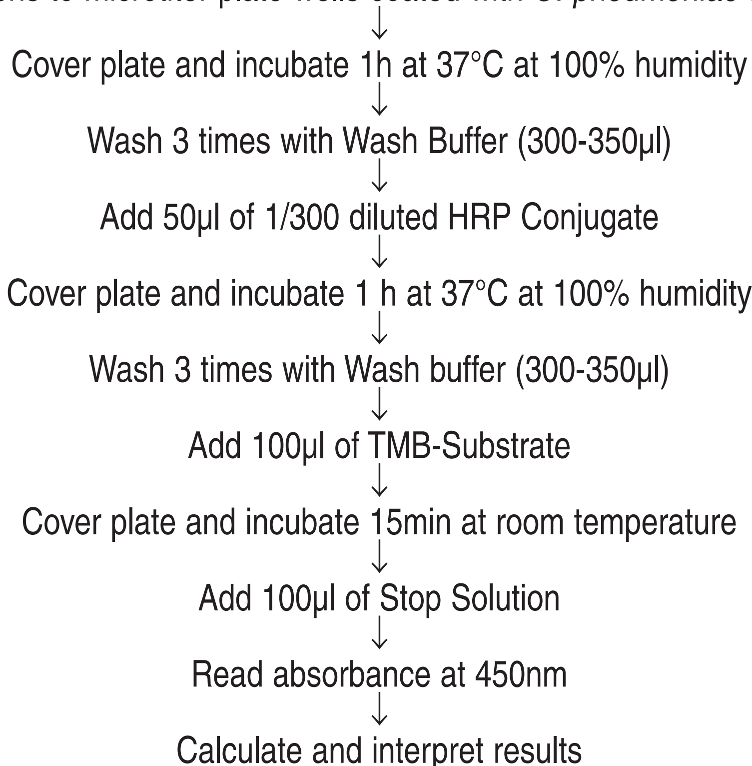
IgA antibodies have shown to be a reliable immunological marker of primary, chronic and recurrent infections. These antibodies usually decline rapidly to baseline levels following treatment and eradication of the chlamydia infections (3). The persistence of elevated IgA antibody titers is generally considered as a sign of chronic infection (6).
IgG antibodies persist for long periods and decline very slowly. Therefore, the presence of IgG antibodies is mainly indicative of a chlamydia infection at an undetermined time. However, a four-fold rise in IgG or high levels of IgG antibodies may indicate an on-going chronic infection.

SeroCP™ is an ELISA based assay in which purified elementary bodies of *C. pneumonaie* (TWAR-183) are used as antigens to detect the antibody response in humans. For complete diagnosis of current, chronic or past infections, it is recommended to determine IgG, IgM and IgA antibodies to *C. pneumoniae*.

Principle of the Test

- SeroCP™ Plates are supplied coated with purified elementary bodies of *C.pneumoniae* (TWAR 183) antigens.
- The serum to be tested is diluted and incubated in the SeroCP™ plate for 1h at 37°C. In this step *C.pneumoniae* antibodies are bound to the immobilized antigens.
- 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 that are bound to the coated antigens.

Assay Procedure



Kit contents

Test kit for 96 Determinations Cat. No. A191-01M

1. ***C.pneumoniae* antigen-coated microtiter plate:** 96 break-apart wells (8x12) coated with *C.pneumoniae* antigens, packed in an aluminum pouch containing a desiccant card.
   1 Plate
2. **Concentrated Wash Buffer (20X):** A PBS - Tween buffer.
   1 Bottle, 100ml
3. **Serum Diluent (blue):** A ready to use buffer solution. Contains less than 0.05% proclin as a preservative.
   1 Bottle, 30ml
4. **Conjugate Diluent (green):** A ready to use buffer solution. Contains less than 0.05% proclin as a preservative.
   1 Bottle, 40ml
5. **Negative Control:** A ready to use *C.pneumoniae* IgG negative human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
   1 Vial, 2.5ml
6. **Positive Control:** A ready to use *C.pneumoniae* IgG positive human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
   1 Vial 2.0ml
7. **Concentrated HRP-Conjugate (300X):** Horseradish Peroxidase (HRP) conjugated anti-human IgG (gamma chain specific). Contains less than 0.05% proclin as a preservative.
   1 Vial, 0.2ml
8. **TMB-Substrate:** A ready to use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.
   1 Bottle, 14ml
9. **Stop Solution:** A ready to use solution. Contains 1M H_2SO_4.
   1 Bottle, 15ml
10. **Plate Cover:** **1 unit**
11. **Instruction Manual:** **1**

Test kit for 192 Determinations Cat. No. B191-01M

1. ***C. pneumoniae* antigen-coated microtiter plate:** 96 break-apart wells (8x12) coated with *C.pneumoniae* antigens, packed in an aluminum pouch containing a desiccant card.
   2 Plates
2. **Concentrated Wash Buffer (20X):** A PBS - Tween buffer.
   2 Bottles, 100ml
3. **Serum Diluent (blue):** A ready to use buffer solution. Contains less 0.05% proclin as a preservative.
   1 Bottle, 60ml

4. **Conjugate Diluent (green):** A ready to use buffer solution. Contains less than 0.05% proclin as a preservative.
1 Bottle, 80ml
5. **Negative Control:** A ready to use *C.pneumoniae* IgG negative human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
1 Vial, 2.4ml
6. **Positive Control:** A ready-to-use *C. pneumoniae* IgG positive human serum. Contains less than 0.05% proclin and less than 0.1% Sodium Azide as preservatives.
1 Vial, 1.25ml
7. **Concentrated HRP-Conjugate (300X):** Horseradish Peroxidase (HRP) conjugated anti-human IgG (gamma chain specific). Contains less than 0.05% proclin as a preservative.
1 Vial, 0.2ml
8. TMB-Substrate: A ready to use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.
1 Bottle, 24ml
9. **Stop Solution:** A ready to use solution. Contains 1M H_2SO_4.
1 Bottle, 30ml
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- conjugate.
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 a 37°C incubator.
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. 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.
4. Diluted sulfuric acid (1M H_2SO_4) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.).

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 up to twenty-one days.

Serum Collection

Prepare sera from aseptically collected samples using standard techniques. Heat inactivated sera should not be used. The use of lipemic, turbid or 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.

Specimens 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 1/105 with the supplied Serum Diluent as follows: Add 10µl of patient serum to 200µl of Serum Diluent (1/21) and then dilute further by adding 25µl of the 1/21 dilution to 100µl of Serum Diluent.
6. Dispense 50µl of Positive control, Negative control and 1/105 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-conjugated anti-human IgG should be diluted to working solution shortly before use. Dilute the concentrated HRP-conjugated anti-human IgG 1/300 with conjugate diluent For example, for two strips prepare a minimum of 3ml of diluted HRP-conjugate (10µl of Concentrated HRP-conjugated anti-human IgG is mixed with 3ml of Conjugate Diluent).
12. Dispense 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. Dispense 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 should be: 0.1< NC ≤ 0.4 at 450nm.

Calculation of Cut-Off Value (COV) and of 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 is calculated according to the following formula:

$$\text{COI} = \frac{\text{Serum sample absorbance at 450nm}}{\text{COV}}$$

Interpretation of Results

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

Absorbance (450nm)	COI	Results	Diagnostic Interpretation
O.D < COV	<1.0	**Negative**	no detectable IgG antibodies No indication of current infection by *C.pneumoniae*
COV ≤ **O.D** ≤ 1.1xCOV	1-1.1	**Borderline**	low level of IgG antibodies Indication of possible exposure to *C.pneumoniae*. Second sample testing required after 2-4 weeks[1]
O.D >1.1 x COV	>1.1	**Positive**	relevant levels of IgG antibodies Indication of current or past infection by *C.pneumoniae*[2]

1. When testing a second sample, both the first and the second sample should be tested simultaneously. If borderline result is repeated, specimen must be considered negative.

2. In order to differentiate between past or current infection, it is recommended to take a second sample after 2-4 weeks.
   If the COI of the second sample increases by at least 40%, current infection is indicated.

<u>In order to achieve a more comprehensive antibodies' profile, IgM and IgA should also be tested</u>

Table 2: Interpretation of results based on the combination of IgG, IgA and IgM antibodies.

Levels of C. pneumoniae antibodies			Interpretation of Results
IgM	**IgG**	**IgA**	
Negative	**Negative**	Negative	No indication of *C. pneumoniae* infection
Positive	**Negative or Positive**	Negative or Positive	Indication of current infection
Negative	**Positive**	Negative	Indication of past or current infection.
Negative	**Positive or Negative**	Positive	Indication of current 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 2-4 weeks later and tested in parallel with the original sample.

Performance Characteristics

Table 3: Comparison of SeroCP™ IgG with in-house microimmunofluorescence test (MIF)

The seroCP™ IgG was evaluated against in-house MIF test.
The study was carried out at a medical center using 63 sera samples from symptomatic patients and 35 sera samples from healthy individuals.

MIF / SeroCP™	Positive	Negative	Total
Positive	60	1	61
Negative	3	34	37
Total	63	35	98

Sensitivity: 60/63 x 100 = 95%
Specificity: 34/35 x 100 = 97%
Overall agreement: 94/98 x 100 = 96%

Precision

Intra-assay (within-run)

Sample	No. of Replicates	Mean Value	CV%
Positive	10	1.196	3.8
Negative	10	0.160	4.6

Inter-assay (between-run)

Sample	No. of Replicates	Mean Value	CV%
Positive	10	1.152	5.9
Negative	10	0.165	6.4

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SAVYON DIAGNOSTICS Ltd.
3 Habosem St. Ashdod 77610, Israel
Tel: 972.8.8562920 Fax: 972.8.8523176
e-mail: support@savyondiagnostics.com

Obelis s.a. (European Authorized Representative)
Boulevard Général Wahis 53, 1030 Brussels, Belgium
Tel.: +32.2.732.59.54 Fax.: +32.2.732.60.03
e-mail: mail@obelis.net