CE 0483

# SeroCP™ IgG (RT)

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

**Instruction Manual**

Test kit for 96 determinations
REF: 1191-01

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

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## Intended Use

The SeroCP™ IgG (RT) kit is intended for the detection of IgG antibodies specific to *Chlamydia pneumoniae* in human serum.
Savyon® SeroCP™ IgG (RT) kit is a qualitative Enzyme Linked Immunosorbent Assay (ELISA) which is used as an aid in the diagnosis of *Chlamydia pneumoniae* infection.
Savyon® SeroCP™ IgG (RT) is a new configuration of ELISA test which presents advantageous features: incubations at ambient temperature; short test duration; and utilizing ready-to-use conjugate.

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

## Introduction

*Chlamydia pneumoniae* (TWAR183) 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 but increases sharply until middle age, where after it remains high (>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™ RT 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™ RT Plates are coated with *C. pneumoniae* specific antigens.
- Serum to be tested is diluted and incubated with the pre-coated SeroCP™ RT plate 30 minutes at Room Temperature (RT). In this step *C. pneumoniae* specific antibodies are bound to the immobilized *C. pneumoniae* specific antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgG conjugated to horseradish peroxidase (HRP) is added and incubated 30 minutes at Room Temperature. In this step the HRP-conjugate is bound to the pre-formed 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 450/620 nm.
- The absorbance is proportional to the amount of the specific antibodies which are bound to the immobilized *C. pneumoniae* specific antigens.

## Summary of Procedure: Manual/Automation

***Manual procedure:***

Wells of microtiter plate coated with *C. pneumoniae* antigens
↓
Add 2 x 50µl of Cut-Off Control
Add 1 x 50µl each of Negative Control, Positive Control and diluted specimens
↓
Cover plate and incubate 30 minutes at Room Temperature.
↓
Wash 5 times with Wash Buffer
↓
Add 50µl of Ready to Use HRP-Conjugate
↓
Cover plate and incubate 30 minutes at Room Temperature
↓
Wash 5 times with Wash buffer
↓
Add 100µl of TMB-Substrate
↓
Cover plate and incubate 30min at room temperature
↓
Add 100µl of Stop Solution
↓
Read absorbance at 450/620nm
↓
Calculate and interpret results

***Automation procedure:***

Wells of microtiter plate coated with *C. pneumoniae* antigens
↓
Add 2 x 100µl of Cut-Off Control
Add 1 x 100µl each of Negative Control, Positive Control and diluted specimens
↓
Incubate plate 20 minutes at Room Temperature
↓
Wash 5 times with Wash Buffer
↓
Add 50µl of Ready to Use HRP-Conjugate
↓
Incubate plate 30 minutes at Room Temperature
↓
Wash 5 times with Wash buffer
↓
Add 100µl of TMB-Substrate
↓
Incubate plate 30 min at room temperature
↓
Add 100µl of Stop Solution
↓
Read absorbance at 450/620nm
↓
Calculate and interpret results

## Kit contents: for Manual / Automation use

**Test Kit of 96 determinations**
**Catalog No.: A1191-01M / A1191-01D**

1. ***C. pneumoniae* antigen-coated microtiter plate:** 96 break-apart wells (8x12) coated with *C. pneumoniae* specific antigens, packed in an aluminum pouch containing a desiccant card.
   **1 Plate / 1 Plate**
2. **Concentrated Wash Buffer (20X):** A PBS - Tween buffer.
   **1 bottle, 100 ml / 1 bottle, 100 ml**
3. **Serum Diluent-RT (Blue):** A ready to use buffer solution. Contains less than 0.05% Proclin as a preservative.
   **1 Bottle, 30 ml / 2 Bottle, 60 ml**
4. **Ready to Use HRP-Conjugate (Green):** Horseradish Peroxidase (HRP) conjugated anti-human IgG (gamma chain specific). Contains less than 0.05% Proclin as a preservative.
   **1 bottle, 10 ml**
5. **Cut-Off Control**: A ready to use *C. pneumoniae* IgG serum used for cut-off determination. Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.
   **1 Vial, 2.5 ml / 1 Vial, 10ml**
6. **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 ml / 1 Vial, 2 ml**
7. **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 ml / 1 Vial, 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 / 1 Bottle, 16 ml**
9. **Stop Solution:** A ready to use solution. Contains 1M $H_2SO_4$
   **1 Bottle, 15 ml / 1 Bottle, 16 ml**
10. **Plate Cover:** **1 unit / none**
11. **Instruction Manual:** **1 / 1**

## Materials Required But Not Supplied:

1. Clean test tubes for dilution of patients' sera.
2. Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
3. One liter volumetric flask.
4. One 50ml volumetric cylinder.
5. Wash bottle.
6. Absorbent paper.
7. Vortex mixer.
8. ELISA-reader with 450/620nm filter.
9. 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 antibodies to HCV and to HIV 1 & 2. 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 $H_2SO_4$) is an irritant agent for the eyes and skin. In case of contact with eyes, immediately flush area with water and consult a physician.
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, its 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 21 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.

## 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 for Manual Use

***The procedure below is for manual use, please see the following section for automation use***

**A. Preparation of Reagents**

1. Bring all components and clinical specimens to be tested to room temperature. Mix well the Cut-Off Control, Negative Control, Positive 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 Cut-Off Control, and one well of each Negative Control and 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 1/110 with the supplied Serum Diluent-RT as follows: Add 10µl of patient serum to 1090µl of Serum Diluent.
6. Pipette Cut-Off Control in duplicate: 50µl into each well. Pipette 50µl of each: Negative Control, Positive control, and 1/110 diluted sera into separate wells of the test strip.
7. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22ºC-28ºC).
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 four times, for a total of five washing steps.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

**C. Incubation with Conjugate**

11. Remove the appropriate volume of conjugate to be used (50ul/well x number of wells) into a clean disposable reservoir.
12. Pipette 50µl of ready to use HRP Conjugate into each well.
13. Discard any HRP Conjugate remaining (or leftovers).
14. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22ºC-28ºC).
15. Discard the liquid content and wash as described in steps 9-10.

**D. Incubation with TMB - Substrate**

16. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature (22ºC-28ºC) for **30 minutes**.
17. Stop the reaction by adding 100µl of Stop Solution (1M $H_2SO_4$) to each well.

## E. Determination of Results

18. Determine the absorbance at 450/620nm 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 Procedure for Automation Use

***The vials and reagents' volume have been adapted for automation applications.***

### A. Preparation of Reagents

1. Bring all components and the clinical specimens to be tested to room temperature. Mix well the Cut-Off Control, Negative Control, Positive 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 Cut-Off Control and one well of each Negative Control and 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 1/110 as follows: Dispense 1090µl of Serum Diluent-RT to each sample's tube. Add 10µl patient serum to each sample's tube.
6. Pipette Cut-Off Control in duplicate: 100µl into each well. Pipette 100µl of each: Negative Control, Positive control, and 1/110 diluted sera into separate wells of the test strip.
7. Incubate for 20 minutes at room temperature (22-28°C).
8. ***Eliminate assay drift caused by this operation.***
9. **Washing step:** Perform 5 X 500µl wash cycles using Savyon Wash Buffer.
10. Perform 2 aspirate cycles with aspirate sweep.

### C. Incubation with conjugate

11. Remove the appropriate volume of conjugate to be used (50ul/well x number of wells) into a clean disposable reservoir.
12. Pipette 50µl of ready to use HRP Conjugate into each well.
13. Discard any HRP Conjugate remaining (or leftovers).
14. Cover the strips with a plate cover and incubate for 30 minutes at room temperature (22ºC-28ºC).
15. Discard the liquid content and wash as described in steps 9-10.

#### D. Incubation with TMB - Substrate

16. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature (22ºC-28ºC) for **30 minutes**.
17. Stop the reaction by adding 100µl of Stop Solution (1M $H_2SO_4$) to each well.

### E. Determination of Results

18. Determine the absorbance at 450/620nm and record the results. Determination should not exceed 30 minutes following stopping of chromogenic reaction.

***Please note that each automation machine has specific technical commands. Please implement Savyon automation procedure for this kit in the operation protocol of your automation machine.***

## 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.** O.D. $_{\text{Positive Control}} \geq 0.8$
**2.** Ratio O.D.$_{\text{Positive Control}}$/ O.D. $_{\text{Cut Off Control}} > 2$
**3.** O.D.$_{\text{negative control}} < 0.3$

## Calculation of Test Results

1. The average absorbance value of the Cut off serum run in duplicate should be calculated.
2. In order to normalize the results obtained in different tests, the cut off index (COI) is calculated according to the following formula:

$$\text{COI} = \frac{\text{O.D. of the Serum Sample}}{\text{O.D. Average of Cut Off Control}}$$

## Interpretation of Results

**Table 1**

| COI | Results | Diagnostic Interpretation |
|---|---|---|
| <1.0 | **Negative** no detectable IgG antibodies | **No indication of current infection by *C.pneumoniae*** |
| 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] |
| >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.
2. In order to differentiate between past or current infection, it is recommended to take a second sample after 2-4 weeks.

**<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 |

## Performance Characteristics

**Comparison of SeroCP IgG (RT) with SeroCP Quant IgG**

The SeroCP IgG (RT) was evaluated against SeroCP Quant IgG (Savyon Diagnostics, Catalog No. A292-01)

The study used 48 sera samples from symptomatic individuals and 96 sera samples from healthy individuals.

Sensitivity and specificity were calculated:

Sensitivity: 40/44 = 91.0%
Specificity: 44/47 = 93.6%

## Precision

**Table 3:** Intra-assay (within-run) precision of the SeroCP™ IgG (RT) test is shown below:

| Sample | No. of Replicates | Mean Value | CV% |
|---|---|---|---|
| Positive | 12 | 1.143 | 3.74 |
| Negative | 12 | 0.219 | 3.95 |

**Table 4:** Inter-assay (between-run) precision of the SeroCP™– IgG (RT) test is shown below:

| Sample | No. of Replicates | Mean Value | CV% |
|---|---|---|---|
| Positive | 12 | 1.483 | 4.04 |
| Negative | 12 | 0.260 | 8.39 |

## 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.
3. Interfering substances: 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.

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CE 0483


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