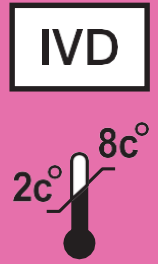


SeroCP™ IgM

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



For professional use only

 0483

SeroCP_IgM Test Kit (ELISA) Instructions for Use



This package insert must be read carefully prior to use and should be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

REF FA192-01

96 Tests per Kit

For In Vitro Diagnostic (IVD) Use

For Professional Use Only **CE** **IVD**



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1. INTENDED USE

SeroCP™ IgM kit is intended for the detection of IgM antibodies specific to Chlamydia pneumoniae in human serum.

The SeroCP™ IgM kit is a qualitative Enzyme Linked Immunosorbent assay (ELISA), which is used as an aid in the diagnosis of Chlamydia pneumoniae infection.

The test enables early diagnosis of current infection in a single serum specimen by detection of IgM antibodies.

For **In Vitro** Diagnostic Use.

Testing with the SeroCP™ IgM Test Kit (ELISA) is intended for use by qualified laboratory personnel specifically instructed and trained in the techniques of ELISA and in vitro diagnostic procedures.

2. SUMMARY AND EXPLANATION

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 pharyngitis, 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.

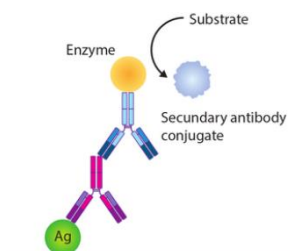
SeroCP™ is an ELISA based assay in which purified elementary bodies of *C. pneumoniae* (TWAR-183) are used as antigens to detect the antibody response in humans. For complete diagnosis of current, chronic or past infections, it is recommended to determine IgG, IgM and IgA antibodies to *C. pneumoniae*.

3. PRINCIPLE OF THE PROCEDURES

Indirect ELISA is a technique that uses a two-step process for detection, whereby a primary antibody specific for the antigen binds to the target, and a labeled secondary antibody against the host species of the primary antibody binds to the primary antibody for detection.

- 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 IgM 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.



4. REAGENTS AND MATERIALS SUPPLIED

96 reactions. REF# FA192-01

Item	Amount	Storage Condition (Before Preparation)
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	96 Wells	2-8°C
Concentrated Wash Buffer (20X): A PBS - Tween buffer.	1 Bottle, 100ml	2-8°C
Serum Diluent (blue): A ready to use buffer solution. Contains 0.05% proclin as a preservative.	2 Bottles, 60ml	2-8°C
Conjugate Diluent (green): A ready to use buffer solution. Contains 0.05% proclin as a preservative.	2 Bottles, 20ml	2-8°C
Negative Control: A ready to use C.pneumoniae IgM negative human serum.	1 Vial, 2.5ml	2-8°C
Positive Control: A ready to use C.pneumoniae IgM positive human serum.	1 Vial 2.0ml	2-8°C
Concentrated HRP-Conjugate (300X): Horseradish Peroxidase (HRP) conjugated anti-human IgM (mu chain specific)	1 Vial, 0.2ml	2-8°C
TMB-Substrate: A ready to use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.	1 Bottle, 16ml	2-8°C
Stop Solution: A ready to use solution. Contains 1M H ₂ SO ₄ .	1 Bottle, 16ml	2-8°C

5. MATERIALS REQUIRED BUT NOT PROVIDED

- Clean test tubes for dilution of patients' sera.
- Disposable plastic vial for dilution of the concentrated HRP- conjugate.
- Adjustable micropipettes, or multichannel pipettes (5-50, 50-200 and 200-1000µl ranges) and disposable tips.
- One-liter volumetric flask.
- One 50ml volumetric cylinder.
- Wash bottle.
- Absorbent paper.
- Vortex mixer.
- A 37°C water bath with a lid, or a moisture chamber placed in a 37°C incubator.
- ELISA-reader with 450nm filter.
- Distilled or double deionized water.

6. STORAGE AND EXPIRATION DATE

- 6.1 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!
- 6.2 Once the kit is opened, its shelf life is 90 days.
- 6.3 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.
- 6.4 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.

7. WARNING AND PRECAUTIONS

Please read these instructions carefully prior to beginning the assay.

For In Vitro Diagnostic Use

- 7.1 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.

7.2 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.

7.3 Safety note for reagents containing hazardous substances:

7.3.1 Stop solution (1M H₂SO₄)

Therefore, the following hazard and precautionary statements apply:

Danger



Hazard statement(s)

H290 May be corrosive to metals.

H314 Causes severe skin burns and eye damage.

Precautionary statement(s)

P234 Keep only in original packaging.

P280 Wear protective gloves/ protective clothing/ eye protection/ face protection.

P303 + P361 + P353 IF ON SKIN (or hair): Take off immediately all contaminated clothing. Rinse skin with water.

P304 + P340 + P310 IF INHALED: Remove person to fresh air and keep comfortable for breathing. Immediately call a POISON CENTER/ doctor.

P305 + P351 + P338 IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing.

P363 Wash contaminated clothing before reuse.

7.3.2 Reagents contain ProClin 300 (refer section 4 for details) as preservatives less than 0.05%.

The following hazard and precautionary statements apply:

Warning



Warning H317 May cause an allergic skin reaction.

P261 Avoid breathing spray

P280 Wear protective gloves/ protective clothing.

P302+P352 IF ON SKIN: Wash with plenty of soap and water.

P333+P313 If skin irritation or rash occurs: Get medical advice/ attention.

8. SAMPLE REQUIREMENTS

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.

SAMPLE PREPARATION

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.

9. TECHNICAL HINTS

Avoid foaming or bubbles when mixing or reconstituting components

- Avoid cross contamination of samples or reagents by changing tips between sample, standard and reagent additions.
- Ensure plates are properly sealed or covered during incubation steps
- Complete removal of all solutions and buffers during wash steps is necessary for accurate measurement readings

10. TEST PROCEDURE - MANUAL

10.1 Reagent Preparation (Solution Preparation Area)

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. 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.
3. 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.

10.2 Incubation of sera samples and controls

1. **One step Dilution:** Dilute each patient serum 1/105 with the supplied Serum Diluent as follows: Add 5µl of patient serum to 520µl of Serum Diluent.

Two Steps Dilution: 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 1/21 dilution to 100µl of Serum Diluent.

Note: The serum diluent contains anti-human IgG, for the removal of IgG antibodies from human serum

2. 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.**
3. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
4. Discard the liquid content of the wells.
5. **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.
6. Dry the strips and frame by gently tapping them over clean absorbent paper.

10.3 Incubation with Conjugate

1. Concentrated HRP-conjugated anti-human IgM should be diluted to working solution shortly before use. Dilute the concentrated HRP-conjugated anti- human IgM 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 IgM is mixed with 3ml of Conjugate Diluent).
2. Dispense 50µl of diluted conjugate into each well.
3. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
4. Discard the liquid content and wash as described in steps 10.2 (5-6).

10.4 Incubation with TMB - Substrate

1. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for 15 minutes.
2. Stop the reaction by adding 100µl of Stop Solution (1M H₂SO₄) to each well.

10.5 Determination of Results

1. 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.

Summary of Procedure:

Add 2x50µl of Negative Control, 1x50µl of Positive Control and 1/105 diluted specimens to microtiter plate wells coated with *C. pneumoniae* antigens

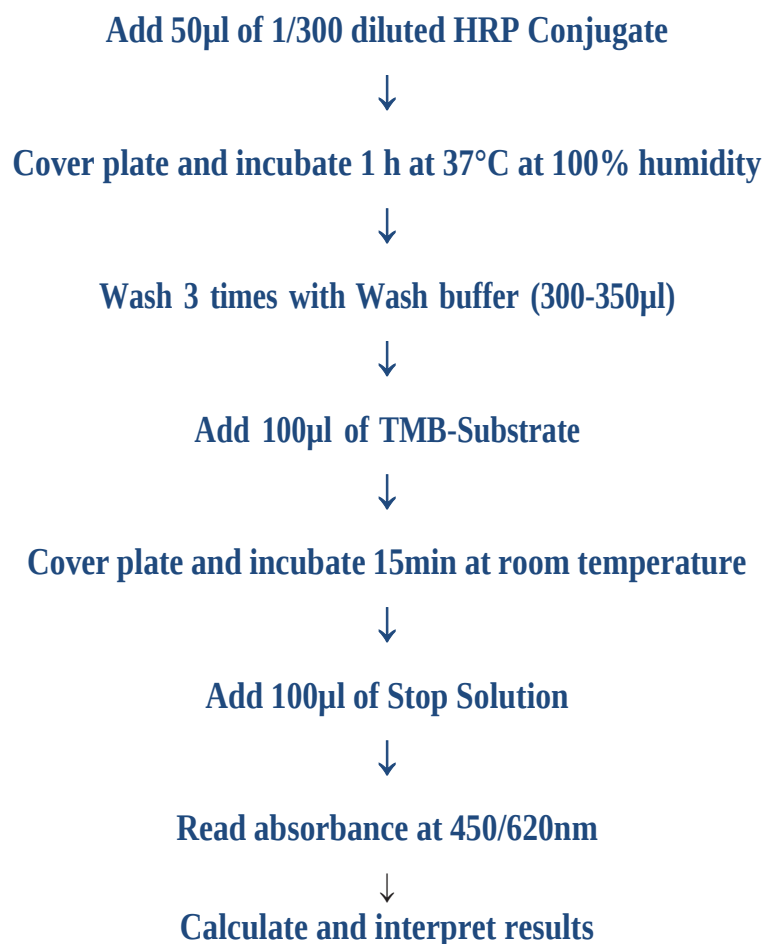


Cover plate and incubate 1h at 37°C at 100% humidity



Wash 3 times with Wash Buffer (300-350µl)





11. QUALITY CONTROL/ASSAY CONTROL RESULTS

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:
 $0.1 < NC \leq 0.4$ 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:

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

12. INTERPRETATION OF PATIENT RESULTS

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

Absorbance (450nm)	COI	Results	Diagnostic Interpretation
$O.D < 1.4COV$	<1.4	Negative	No indication of current infection by <i>C.pneumoniae</i>
$1.4COV \leq O.D \leq 1.5xCOV$	1.4-1.5	Borderline	Indication of possible exposure to <i>C.pneumoniae</i> Second sample testing required after 2-4 weeks ¹
$O.D > 1.5 \times COV$	>1.5	Positive	Indication of current infection by <i>C.pneumoniae</i>

1. In case of borderline results a second serum sample should be taken 2-4 weeks later and tested together with the first sample.
If borderline result is repeated, specimen must be considered negative.

In order to achieve a more comprehensive antibodies' profile, IgG and IgA should also be tested

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

Levels of <i>C. pneumoniae</i> antibodies			Interpretation of Results
IgM	IgG	IgA	
Negative	Negative	Negative	No indication of <i>C. pneumoniae</i> 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

13. TEST LIMITATIONS

1. No single serological test should be used for a final diagnosis. All clinical and laboratory data should be considered.
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.

14. PERFORMANCE CHARACTERISTICS

15.1 Clinical performance

Table 3: Comparison of SeroCP™ IgM with Chlamydia IgM SeroFIA™

The SeroCP™ IgM was evaluated against Chlamydia IgM SeroFIA™ (Savyon Diagnostics Ltd.) with 113 Clinical sera samples from suspected population provided by Biominis

SeroFIA™ SeroCP™	Positive	Negative	Total
Positive	30	4	34
Negative	3	76	79
Total	33	80	113

Sensitivity: $30/33 \times 100 = 91\%$

Specificity: $76/80 \times 100 = 95\%$

Accuracy: $106/113 \times 100 = 94\%$

Precision

Intra-assay (within-run)

Sample	No. of Replicates	Mean Value	CV%
Positive	10	1.469	2.3
Negative	10	0.236	4.7

Inter-assay (between-run)

Sample	No. of Replicates	Mean Value	CV%
Positive	30	0.605	5.4
Negative	30	0.163	6.0

15.2 Analytical Performance

15.2.1 Analytical Sensitivity (Limit of Detection, LoD)

The limit of deduction for SeroCPT™ IgG Test is dilution of 1:3360 antibodies.

15.2.2 Cross-Reactivity

The SeroCPT™ IgG was tested with the following bacteria and there was no cross reactivity and interference with: CMV, Chlamydia Trachomatis and EBV.

15.2.3 Interfering Substances

No interference was observed with the following:

Interferences of 5 positive samples and 5 negative samples (were run on three different batches of SeroCP IgM) are not observed up to a concentration of 5.3mg/Dl, 10.5mg/Dl and 21mg/Dl Bilirubin, concentrations of 235mg/Dl and 470mg/Dl hemoglobin and Triglycerides (TB) in concentrations of 25mg/Dl and 50mg/Dl.

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16. Packaging symbols:

Packaging symbols:	
	Catalogue Number
	In Vitro Diagnostic Medical Device
	Manufacturer
	Store at 2°C – 8°C
	Expiry Date
	Warning
	Danger
	Contains Sufficient for 96 tests
	Consult Instructions for Use
	CE mark
	EU Authorized representative
	Batch Number