savyonDIAGNOSTICS

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REF A293-01M

SeroCP™ Quant IgA

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

IVD

For professional use only

A

CE 0483

SeroCP™ Quant IgA

Intended Use

SeroCP™ Quant IgA kit is an Enzyme Linked Immunosorbent assay (ELISA) for the semi-quantitative determination of species-specific IgA antibodies to *Chlamydia pneumoniae* in human serum.

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

Introduction

Chlamydia pneumoniae (TWAR-183) 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 pharyngititis, 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), and 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 chlamydial 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™ Quant 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™ Quant Plates are supplied coated with purified elementary bodies of *C.pneumoniae* (TWAR 183) as antigens.
- The serum to be tested is diluted and incubated in the SeroCP™ Quant 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 IgA 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 chromogen.
- Upon the addition of the Stop Solution, the blue color turns yellow and should be read by an ELISA reader at a wavelength of 450/620nm.
- The absorbance is proportional to the amount of the specific antibodies that are bound to the coated antigens.

Kit contents

Test kit for 96 Determinations

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, (pH 7.4-7.6) which contains NaCl, Na_2HPO_4, KH_2PO_4 and Tween 20.

   1 bottle, 100ml
3. **Serum Diluent (blue):** A ready-to-use buffer solution. Contains less than 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, 40ml
5. **Negative Control:** A ready-to-use *C. pneumoniae* IgA negative human serum. Contains less than 0.1% Sodium azide as preservative.
1 Vial, 2ml
6. **Positive Control:** A ready-to-use *C.pneumoniae* IgA positive human serum. Contains less than 0.1% Sodium azide as preservative.
1 Vial, 2ml
7. **P10-calibrator:** A ready-to-use *C.pneumoniae* IgA positive human serum contains 10 BU/ml of IgA. Contains less than 0.1% Sodium azide and less than 0.05% Proclin as preservatives.
1 Vial, 2ml
8. **P50-calibrator:** A ready-to-use *C.pneumoniae* IgA positive human serum contains 50 BU/ml of IgA. Contains less than 0.1% Sodium azide and less than 0.05% Proclin as preservatives.
1 Vial, 2ml
9. **P100-calibrator:** A ready-to-use *C.pneumoniae* IgA positive human serum contains 100 BU/ml of IgA. Contains less than 0.1% Sodium azide and less than 0.05% Proclin as preservatives.
1 Vial, 2ml
10. **Concentrated HRP-Conjugate (300X):** Horseradish peroxidase (HRP) conjugated anti-human IgA (alfa chain specific). Contains less than 0.05% Proclin as a preservative.
1 Vial, 0.2ml
11. **TMB-Substrate:** A ready-to-use solution. Contains 3, 3', 5, 5' - tetramethylbenzidine as a chromogen and peroxide as a substrate.
1 Bottle, 14ml
12. **Stop solution:** A ready-to-use 1M H_2SO_4 Solution.
1 Bottle, 15ml
13. **Plate Cover:** **1 unit**
14. **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 (5-50, 50-200 and 200-1000µl ranges) or multichannel pipettes and disposable tips.
4. One liter volumetric flask.
5. One 50ml volumetric cylinder.
6. Microplate washer or a 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 a 450 and 620nm filters.
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 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".
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, 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 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.

Storage

Specimens should be stored at 2-8°C and tested within 7 days (adding of 0.1% Sodium Azide is highly recommended). If 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 the clinical specimens to be tested to room temperature. Mix well the calibrators (P10, P50, P100), Negative and Positive Controls 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: One well of Positive Control, one well of Negative Control and three wells of calibrators (P10, P50, P100).
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 1:400 with the supplied Serum Diluent by using one of the following options:
   a. **Recommended for the use of an automated system: (This method requires an extra bottle of Serum Diluent)**
   - Add 10µl of patient serum to 990µl of Serum Diluent (1:100).
   - Dispense 45µl of Serum Diluent to each well of the test strip. Add 15µl of the 1:100 pre-diluted sample to each well.
   - Dispense 60µl of each of the ready to use Positive Control, Negative Control and 3 calibrators (P10, P50, P100) into separate wells.

   b. **Recommended for the use of a manual system:**
   - Add 10µl of patient serum to 190µl of Serum Diluent (1:20).
   - Dilute further by adding 10ml of 1:20 dilution to 190µl of Serum Diluent.
   - Dispense 50µl of ready to use Positive Control, Negative Control, 3 calibrators and diluted sera samples into separate wells of the strips.
6. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.
7. Discard the liquid content of the wells.
8. **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.
9. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with conjugate

10. Concentrated HRP-conjugated anti-human IgA should be diluted to working solution shortly before use. Dilute the concentrated HRP-conjugated anti-human IgA 1/300 with conjugate diluent.
    For example: for two strips prepare a minimum of 3ml conjugate as follows: 10µl of Concentrated HRP-conjugated anti-human IgA is mixed with 3ml of Conjugate Diluent.
11. Dispense 50µl of diluted conjugate into each well.
12. Cover the strips with a plate cover and incubate for 1h at 37°C in a moisture chamber.

13. Discard the liquid content and wash as described in steps 8-9.

D. Incubation with TMB - Substrate

14. Dispense 100µl TMB-Substrate into each well, cover the strips with a plate cover and incubate at room temperature for **15 minutes.**
15. Stop the reaction by adding 100µl of Stop Solution (1M H_2SO_4) to each well.

E. Determination of Results

16. 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 Validation

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

1. O.D $_{P100/P10}$ > **3.8**
2. O.D $_{P50/P10}$ > **2.3**
3. NC should be < **10 BU/ml**
4. PC should be > **30 BU/ml**

Calculation of Test Results

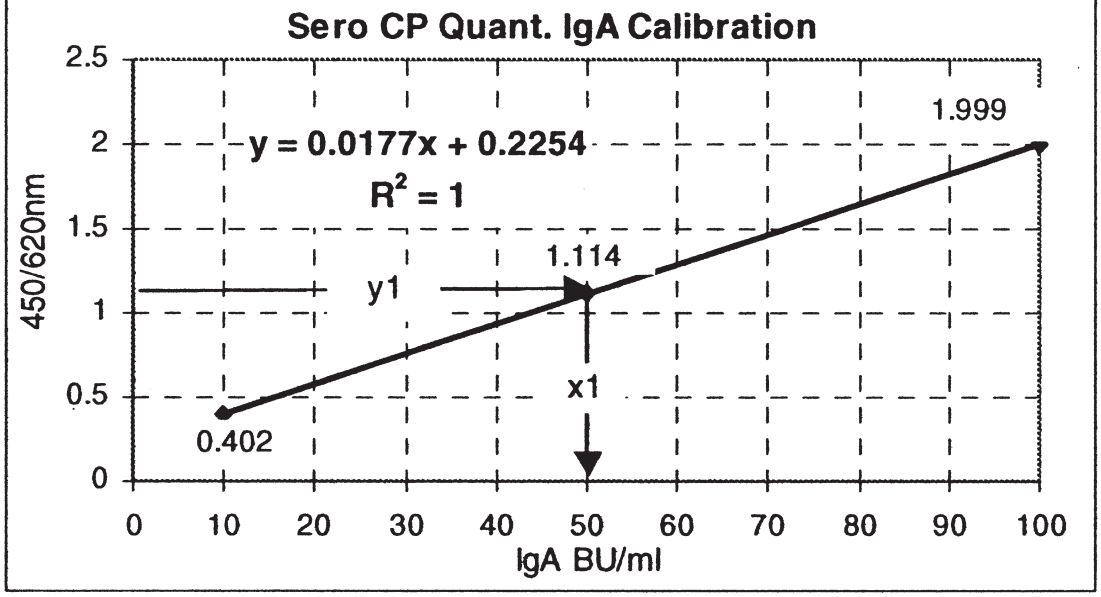
Manual method, using a squared graph paper:

1. Plot the absorbance values (OD) of the 3 calibrators (P10, P50 and P100) on Y axis versus their concentration (BU/ml) on X axis.
2. Draw the best fitted linear curve through the points.
3. Using the standard curve, interpolate the concentration of the tested sample values (in BU/ml) from each absorbance measured (see example 1).

Example 1: Interpolation of results

On the Y-axis read the absorbance value of the sample and draw a horizontal line to the calibration curve.
From the intercept, draw a vertical line to the
X-axis and interpret the concentration in BU/ml of the sample.

Calibrators	IgA BU/ml	OD 450/620nm
P10	10	0.402
P50	50	1.114
P100	100	1.999
Sample	x1=55	y1=1.2



Interpretation of Results

IgA BU/ml	Result	Diagnostic Interpretation
< 10 BU/ml	**Negative** No detectable IgA antibodies	**No indication of *C. pneumoniae* Infection**
≥ 10 BU/ml	**Positive** Relevant level of IgA antibodies	**Indication of current or chronic *C. pneumoniae* infection[1]**

BU: Binding Units

1 **In order to differentiate between current or chronic infection follow up studies are recommended.**

Correlation of SeroCP™ Quant results with SeroFIA™ IgA – MIF End Point Titers (EPT)

SeroCP™ Quant IgA semi-quantitative kits were calibrated in comparison with SeroFIA™ IgA (Savyon Diagnostics Ltd., Israel) MIF system. The correlation is presented in Table no.1.

Table no. 1

SeroCP™ Quant IgA Range of BU/ml	SeroFIA™ IgA End Point Titers
<10	Neg
10-35	32
36-65	64
66-110	128
>110	≥ 256

Note: There is no standardization of different MIF system results. Each MIF test might yield different EPT.

Performance Characteristics

Comparison of SeroCP™ Quant IgA with SeroCP™IgA

The SeroCP™ Quant IgA was evaluated against SeroCP™ IgA (Savyon Diagnostics Ltd., Catalog No. 193-01).
The study used 230 sera samples from symptomatic individuals and 58 sera samples from healthy individuals.

Sensitivity and specificity were calculated:
Sensitivity: 218/230 = 94.8%
Specificity: 54/58 = 93.1%

Cross Reaction

Hospitalized patients, infected with *C. trachomatis*, *CMV* and *EBV*, who were diagnosed by commercial serology kits, were also tested with the SeroCP™ Quant kit. There was no significant cross reaction detected.

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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M293-01E 04-10/13

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