savyonDIAGNOSTICS

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192

SeroMP™ IgA

REF A263-01M
REF B263-01M

IVD

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

For professional use only

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SeroMP™ IgA

Intended Use

SeroMP™ IgA kit is a semi-quantitative Enzyme Linked Immunosorbent assay (ELISA) for the determination of species specific IgA antibodies to *Mycoplasma pneumoniae* in human serum.
Savyon® SeroMP™ IgA kit is used as an aid in the diagnosis of *Mycoplasma pneumoniae* infection.

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

Introduction

M.pneumoniae is a common cause of community-acquired pneumonia, often characterized by gradual onset of headache, fever, malaise and, most typically, dry cough. *M.pneumoniae* is common in all age groups, however, it is most common in the first two decades of life and is rare in children under the age of four. It has been reported as the cause of up to 30% of all pneumonia cases (1).
M.pneumoniae has also been associated with non respiratory diseases as meningitis, encephalitis, pancreatitis, sensorineural hearing loss, and acute brainstem syndrome(2).

Due to its common occurrence, one should consider *M.pneumoniae* in all cases of pneumonia, but being the same symptoms for different agents, additional diagnostic tools, such as serological tests, are required (3).
The ELISA technique is sensitive, specific and enables a differential determination of specific IgG, IgA and IgM antibodies (4).

M.pneumoniae specific IgM antibodies rise early after onset of the disease, reach peak levels in one to four weeks, then decline to diagnostically insignificant levels within a few months (5). Due to the early appearance and relatively short life time of IgM antibodies, their detection allows the diagnosis of acute infection using a single serum sample. Young patients tend to have higher IgM levels than adults (6). IgG levels rise slower than IgM, but remain elevated much longer, so a significant increase in two consecutive samples taken at least 2 weeks apart, may indicate current infection or re-infection even in the absence of IgM. IgA antibodies are seen at higher levels in elderly patients (5) and may be more useful than IgM for the diagnosis of current infection in adults (6).

Savyon® Diagnostics Ltd. has developed semi-quantitative IgG, IgA and IgM ELISA tests which enable to follow the change of antibody levels in human sera. The antigen used in the SeroMP™ test is a membrane preparation of M.pneumoniae that contains the P1 membrane protein, which is a major immunogen (7, 8, 9, 10, 11).

The SeroMP™ test enables early and accurate detection of *M. pneumoniae* specific IgG, IgA and IgM antibodies.

Principle of the Test

- SeroMP™ microtiter plates are supplied coated with purified fraction of *M.pneumoniae* membrane proteins.
- The serum to be tested is diluted and incubated in the SeroMP™ plate. In this step M. pneumoniae specific antibodies are bound to the immobilized antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgA conjugated to horseradish peroxidase (HRP) is added. 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 450/620nm.
- The absorbance is proportional to the levels of the specific antibodies that are bound to the coated antigens.

Assay Procedure

Wells of microtiter plate coated with *M.pneumoniae* antigens

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Add 50µl of Negative Control, 50µl of Positive Control, 50µl of each calibrator: (P10, P50, P75), and diluted specimens

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

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Wash 3 times with Wash Buffer

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Add 50µl of 1/300 diluted HRP Conjugate

↓

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

↓

Wash 3 times with Wash buffer

↓

Add 100µl of TMB-Substrate

↓

Cover plate and incubate 15 min at room temperature

↓

Add 100µl of Stop Solution

↓

Read absorbance at 450/620nm

↓

Calculate and interpret results

Kit contents

Test kit for 96 Determinations — Cat. No. A263-01M

1. ***M. pneumoniae* antigen coated microtiter plate:** 96 break apart wells (8x12) coated with *M.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. **Positive Control:** A ready-to-use *M.pneumoniae* IgA positive human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.

   1 Vial, 2.0ml

6. **Negative Control:** A ready-to-use *M.pneumoniae* IgA negative human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.

1 Vial, 2.0ml

7. **P10-calibrator:** A ready-to-use *M.pneumoniae* IgA low positive human serum. Contains 10 BU/ml of IgA (arbitrary binding units) Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.

1 Vial, 2.0ml

8. **P50-calibrator:** A ready-to-use *M.pneumoniae* IgA medium positive human serum. Contains 50 BU/ml of IgA (arbitrary binding units). Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.

1 Vial, 2.0ml

9. **P75-calibrator:** A ready-to-use *M.pneumoniae* IgA high positive human serum.. Contains 75 BU/ml of IgA (arbitrary binding units). Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.

1 Vial, 2.0ml

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 solution. Contains 1M H_2SO_4.

1 Bottle, 15ml

13. **Plate Cover:** **1 Unit**
14. **Instruction Manual:** **1**

Test kit for 192 Determinations — Cat. No. B263-01M

1. ***M. pneumoniae* antigen coated microtiter plate:** 96 break apart wells (8x12) coated with *M.pneumoniae* antigens, 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 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. **Positive Control:** A ready-to-use *M.pneumoniae* IgA positive human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.

1 Vial, 2.0ml

6. **Negative Control:** A ready-to-use *M.pneumoniae* IgA negative human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.
1 Vial, 2.0ml
7. **P10-calibrator:** A ready-to-use *M.pneumoniae* IgA low positive human serum. Contains 10 BU/ml of IgA (arbitrary binding units) Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.
1 Vial, 2.0ml
8. **P50-calibrator:** A ready-to-use *M.pneumoniae* IgA medium positive human serum. Contains 50 BU/ml of IgA (arbitrary binding units). Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.
1 Vial, 2.0ml
9. **P75-calibrator:** A ready-to-use *M.pneumoniae* IgA high positive human serum.. Contains 75 BU/ml of IgA (arbitrary binding units). Contains less than 0.1% Sodium Azide and less than 0.05% Proclin as preservatives.
1 Vial, 2.0ml
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, 24ml
12. **Stop Solution:** A ready-to-use solution. Contains 1M H_2SO_4.
1 Bottle, 30ml
13. **Plate Cover:** **2 Units**
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 and 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 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 1and 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 reagents 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, P75), 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: One well of blank, one well of Negative Control, Positive Control and three wells of calibrators (P10, P50, P75).
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 1/21 dilution to 100µl of Serum Diluent.
6. Dispense 50µl of blank (serum diluent), Negative Control, Positive Control, three calibrators (P10, P50, P75), and 1:105 diluted serum samples into separate wells of the test strip.
7. Cover the strips with a plate cover and incubate for 1h at 37°C at 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 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 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.
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 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. $O.D._{P75}$ > **0.9**
2. Ratio: $O.D._{P10}$ / $O.D._{NC}$ > **1.5**
3. Ratio: $O.D._{P50}$ / $O.D._{NC}$ > **4**
4. Ratio: $O.D._{P75}$ / $O.D._{NC}$ > **5.5**
5. PC should be ≥ **40 BU/ml**

Calculation of Test Results

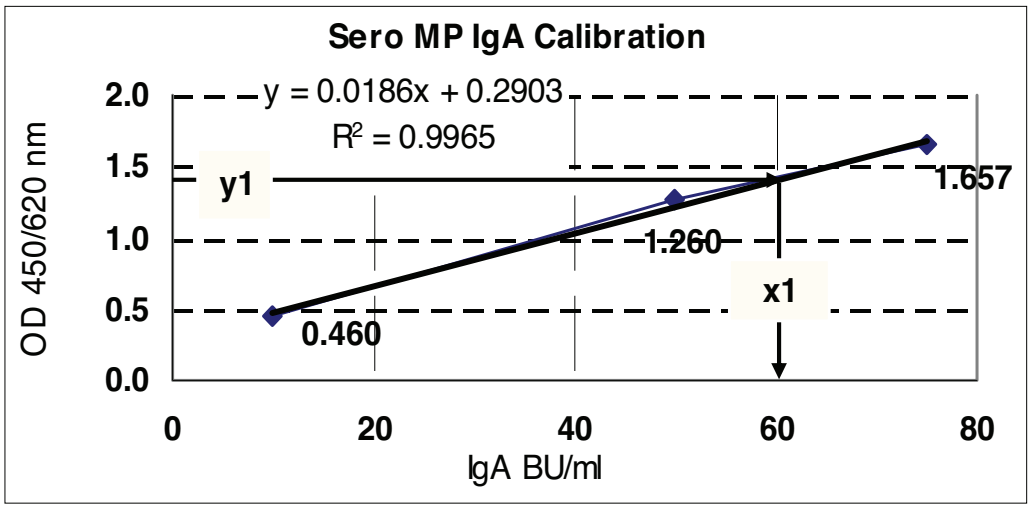
Manual method, using a squared graph paper:

1. Plot the absorbance values (OD) of the 3 calibrators (P10, P50 and P75) 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 (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.
Read the concentration in BU/ml of the sample.

Calibrators	IgA BU/ml	OD 450/620 nm
P10	10	0.460
P50	50	1.260
P75	75	1.657
Sample	x1=60	y1=1.408



Interpretation of Results

IgA BU/ml	Result	Diagnostic Interpretation
< 10 BU/ml	**Negative** No detectable IgA antibodies	**No indication of *M. pneumoniae* infection**
≥ 10 BU/ml ≤ 20 BU/ml	**Borderline**	**Test a second sample, drawn two to four weeks later in parallel with the first sample. When second sample is borderline the result should be considered as negative**
> 20 BU/ml	**Positive** Relevant level of IgA antibodies	**Indication of current or chronic *M. pneumoniae* infection**

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

Interpretation of results based on the combination of IgG and IgM and IgA antibodies detection.

Level of *M. pneumoniae* antibodies			
IgG	**IgM**	**IgA**	
Negative	Negative	**Negative**	**No indication of *M. pneumoniae* infection**
Negative or Positive	Positive	**Negative or Positive**	**Indication of current infection**
Positive	Negative	**Negative**	**Indication of past infection**
Negative or Positive	Negative	**Positive**	**Indication of current infection or re-infection**

Cross Reaction

Hospitalized patients, infected with respiratory tract pathogens: *Chlamydia pneumoniae, Influenza A., Influenza B., Parainfluenza* 1, 2 and 3 as well as *Adenovirus* and *EBV* who were diagnosed by commercial serology kits, were also tested with the SeroMP kit. Most of the sera were found negative, there was no significant cross-reaction detected.

Test Limitations

1. No single serological test should be used for 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 Mycoplasma 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.

Performance Characteristics

Sensitivity and Specificity

A study using SeroMP™ IgA and another commercial ELISA assay was performed on 13 sera obtained from hospitalized pneumoniae patients and 98 sera obtained from healthy blood donors.

Commerical ELISA / SeroMP™	Positive	Negative	Total
Positive	12	7	19
Negative	1	91	92
Total	13	98	111

Sensitivity: 12/13 x 100 = 92%
Specificity: 91/98 x 100 = 93%
Overall Agreement: 103/111 x 100 = 93%

Precision

Intra-assay (within-run) precision

Sample	No of Replicates	Mean Value	CV%
Positive	10	1.390	5.9%
Negative	10	0.314	4%

Inter-assay (between-run) precision:

Sample	No of Replicates	Mean Value	CV%
Positive	10	1.300	6.3%
Negative	10	0.285	7.2%

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