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SeroMP™ Recombinant IgM

Enzyme -Linked Immunosorbent Assay (ELISA)
for the qualitative detection
of specific IgM antibodies to
Mycoplasma pneumoniae
in human serum

Instruction Manual

Test kit for 96 determinations
(Catalog No. 1262-01)

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

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Intended Use

SeroMP™ Recombinant IgM kit is a qualitative Enzyme Linked Immunosorbent assay (ELISA) for the detection of IgM antibodies specific to *Mycoplasma pneumoniae* in human serum.
The test enables early diagnosis of current or acute infection in a single serum specimen by determination of IgM antibodies.

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 (2).
M. pneumoniae has also been associated with non respiratory diseases as meningitis, encephalitis, pancreatitis, sensorineural hearing loss, and acute brainstem syndrome (5).
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 (6).
In respect to diagnosis and treatment, the most prominent structural feature of MP is the lack of a cell wall. It has been shown that surface-exposed polypeptides elicit an immunogenic response, in particular those that are involved in the attachment organelle of MP. This attachment organelle is composed of a complex of polypeptides, in which P1 Cytadhesin Protein has a major role. (1; 4; 10) Due to its high immunogenicity P1 is a paradigm for utilizing a definitive antigen in serology-based diagnostic systems, attempting to improve various parameters of assay performance. A common way to improve test performances by using highly immunogenic polypeptides like the P1 is incorporating these polypeptides in the tests as recombinant antigens. Indeed, several polypeptides have been identified in the literature as good candidates for this purpose. (9)
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 (7). Due to the early appearance and relatively short lifetime 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 (8). 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 (7) and may be more useful than IgM for the diagnosis of current infection in adults (8).
Savyon® Diagnostics Ltd. has developed semi-quantitative kits utilising recombinant antigens in IgG, IgA ELISA tests and a qualitative kit utilising mixture of recombinant and native antigen in the IgM ELISA test which enable to follow the change of antibody levels in human sera.
The SeroMP™ Recombinant IgG, IgA and IgM tests enable early and accurate detection of *M. pneumoniae* infection.

Principle of the Test

- SeroMP™ Recombinant microtiter plates are supplied coated with purified fraction of *M. pneumoniae* membrane proteins and recombinant antigens.
- The serum to be tested is diluted and incubated in the SeroMP™ Recombinant plate. In this step *M. pneumoniae* specific antibodies are bound to the immobilized antigens.
- Non-specific antibodies are removed by washing.
- Anti-human IgM 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
↓
Add 2 x 50µl of Cut Off Control.
Add 1x 50µl of each Negative Control, Positive Control and diluted specimens
↓
Cover plate and incubate 1h at 37ºC at 100% humidity
↓
Wash 3 times with Wash Buffer
↓
Add 50µl of 1/300 diluted HRP Conjugate
↓
Cover plate and incubate 1 h at 37ºC at 100% humidity
↓
Wash 3 times with Wash buffer
↓
Add 100µl of TMB-Substrate
↓
Cover plate and incubate 15min at room temperature
↓
Add 100µl of Stop Solution
↓
Read absorbance at 450/620nm
↓
Calculate and interpret results

Kit Contents

Test kit for 96 Determinations

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 (20 X):** A PBS - Tween buffer.
   1 Bottle, 100 ml
3. **UniDiluent (yellow):** A ready-to-use buffer solution. Contains less than 0.05% Proclin as a preservative.
   1 Bottle, 60 ml
4. **IgM-Serum Diluent (orange):** A ready-to-use Anti-Human IgG in buffer solution. (**IgG/Rf Stripper**) Contains less than 0.05% Proclin as a preservative.
   2 Bottles, 60 ml
5. **Positive Control:** A ready-to-use *M. pneumoniae* IgM positive human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.
   1 Vial, 2.0 ml
6. **Negative Control:** A ready-to-use *M. pneumoniae* IgM negative human serum. Contains less than 0.05% Proclin and less than 0.1% sodium azide as preservatives.
   1 Vial, 2.0 ml
7. **Cut Off Control:** A ready-to-use *M. pneumoniae* IgM 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
8. **Concentrated HRP-Conjugate (300 X):** Horseradish Peroxidase (HRP) conjugated anti-human IgM (µ chain specific).
   1 Vial, 0.2 ml
9. **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
10. **Stop Solution:** A ready-to-use solution. Contains 1M H_2SO_4.
    1 Bottle, 15 ml
11. **Plate Cover:** **1 unit**
12. **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 approved techniques, and found to be negative for HBV antigen and for HCV and HIV 1 and 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, 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.

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

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 1:105 as follows:
   Add 10µl of patient serum to 1040µl of **IgM-Serum Diluent**
   Note: The IgM Serum Diluent contains Anti-human IgG and is used for the removal of IgG antibodies and RF from human serum.
6. Dispense: 50µl of Negative Control; 50µl of Positive Control; Cut Off Control in DUPLICATE; 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 in a moisture chamber.
8. Discard the liquid content of the wells.
9. **Washing step:** Fill each well with wash buffer up to the end of the well and discard the liquid, repeat this step three times.
10. Dry the strips and frame by gently tapping them over clean absorbent paper.

C. Incubation with Conjugate

11. 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 UniDiluent. For example: for two strips prepare a minimum of 3ml conjugate as follows: 10µl of Concentrated HRP-conjugated anti-human IgM is mixed with 3ml of UniDiluent.
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.
    *N**ote:** 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. $_{\text{Positive Control}} \geq$ **1.0**
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:

$$\text{COI} = \frac{\text{OD of the Serum Sample}}{\text{OD Average of Cut Off Control}} \text{ X10}$$

Interpretation of Results

IgM COI	Result	Diagnostic Interpretation
< 10	**Negative** No detectable IgM antibodies	**No indication of *M.Pneumoniae* Infection**
10-11	**Borderline**	**Presence or absence of detectable (Borderline) levels of IgM antibodies to *M.Pneumoniae* cannot be determined. A second serum sample should be obtained after 14-21 days and tested. (When second sample is borderline the result should be considered negative)**
>11	**Positive** Significant level of IgM antibodies	**Indication of current *M.Pneumoniae* infection**

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

Interpretation of results based on the combination of IgM and IgG 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**

Test Performance

Performance of Savyon® SeroMP™ Recombinant test based on IgG, IgA and IgM detection compared to the performance of a respective commercial recombinant based tests*

	Group (N)	SeroMP Recombinant % POS.	Commercial MP% POS.
IgG	Healthy Population (30)	20	40
	Pneumonia Patients (61)	32.8	36.1
IgA	Healthy Population (30)	6.6	3.3, 6.6 (BL)
	Pneumonia Patients (61)	55.7	42.6
IgM	Healthy Population (30)	6.6	3.3
	Pneumonia Patients (56)	64.2	44.6, 18 (BL)

- Higher sensitivity of the SeroMP recombinant in pneumonia patients experiencing acute or chronic infections, as represented by IgM and IgA results
- The prevalence within the healthy population is lower in Savyon Sero MP recombinant assay
- The discrimination between sick patients and healthy population in cases of acute and current infections will be greater in the SeroMP recombinant

*In house study

Cross Reaction

Hospitalized patients, infected with respiratory tract pathogens: *Chlamydia pneumoniae* 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.

Precision

Inter-assay (within-run) precision:

Sample	No. of Replicates	Mean Value	CV%
Positive	10	2.164	4.7
Negative	10	0.159	13.0

Inter-assay (between-run) precision:

Sample	No. of Replicates	Mean Value	CV%
Positive	10	1.196	4.9
Negative	10	0.252	7.3

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.

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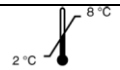	Temperature Limitation
	Consult instructions for use
IVD	In Vitro Diagnostic Medical Device
	Manufacturer
EC REP	Authorized European Representative