EIASON® Mycoplasma pneumoniae IgM

IVD

Enzyme immunoassay for the qualitative determination of IgM-class antibodies against Mycoplasma pneumoniae in human serum or plasma

Instruction for use

For in-vitro use only

Product of

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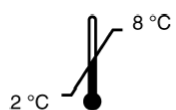

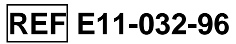

Used IFU-Symbols

Symbol	English	Symbol	English
IVD	In vitro diagnostic device	SUB	Substrate
REF	Order number	MPL	Microplate
	Product of	STOP	Stop solution
2 °C 8 °C	Storage	WASH	Concentrated wash solution
CE	European Conformity	NC	Negative control
	Expiry date	PC	Positive control
LOT	Batch code	CONJ	Conjugate
CUTOFF	CutOff control	DIL	Sample diluent

Intended use

For in-vitro use only.

The EIASON® Mycoplasma pneumoniae IgM kit is intended for the qualitative determination of IgM class antibodies against M. pneumoniae in human serum or plasma (citrate, heparin).

Summary

The mycoplasms belong to the class Mollicutes comprising three distinct families and four genera, one of which is Mycoplasma with over 60 species. Mycoplasmae are the smallest freeliving organisms known (300 to 500 nm in diameter) and unlike regular

bacteria they lack a cell wall. Mycoplasms are extracellular parasites, especially on mucous membranes, which can cause infections in humans, animals, plants, and cell cultures. Mycoplasma pneumoniae is primarily a respiratory pathogen (obligat) in humans involving the nasopharynx, throat, trachea, bronchi, bronchioles, and alveoli. Other Mycoplasmae, M. buccale, M. faucium, M. orale and M. salivarium are commensals in the oral cavity. Mycoplasma hominis and Ureaplasma urealyticum inhabit primarily the genital tract and may act as opportunistic invaders. M. pneumoniae is by far the most important pathogen of this group. Infection with M. pneumoniae occurs worldwide, its epidemiology has been studied primarily in the USA, Europe, and Japan.
Infections are endemic in larger urban areas, and epidemic increases are observed at varying intervalls. Mycoplasma pneumoniae has been estimated to cause 15-20% of all pneumoniae; the rate is highest in children and young adults. 74% of infections with M. pneumoniae are asymptomatic, reinfection may occur. Naturally acquired immunity to infection with M. pneumoniae appears to be of limited duration (2-3 years).

Species	Disease	Symptoms (e.g.)	Transmission route
Mycoplasma pneumoniae	Respiratory diseases by Mycoplasma pneumoniae	Fever, headache, and a persistent cough. Respiratory tract disease: from asymptomatic infection to colds, pharyngitis, bronchitis, croup, tracheobronchitis, pneumonitis and primary atypical pneumonia	Transmitted by aerosol droplets

The presence of pathogen or Infection may be identified by:

- ◊ Microscopy
- ◊ Serology: e.g. by ELISA

Assay principle

The qualitative immunoenzymatic determination of specific antibodies is based on the ELISA (Enzyme-linked Immunosorbent Assay) technique. Microplates are coated with specific antigens to bind corresponding antibodies of the specimen. After washing the wells to remove all unbound sample material a horseradish peroxidase (HRP) labelled conjugate is added. This conjugate binds to the captured antibodies. In a second washing step unbound conjugat is removed. The immune complex formed by the bound conjugate is visualized by adding Tetramethylbenzidine (TMB) substrate which gives a blue reaction product. The intensity of this product is proportional to the amount of specific antibodies in the specimen. Sulphuric acid is added to stop the reaction. This produces a yellow endpoint colour. Absorbance at 450/620 nm is read using an ELISA microwell plate reader.

Warnings and precautions

The EIASON® Mycoplasma pneumoniae IgM kit is for in vitro diagnostic use only and is not for internal use in humans or animals.
This product must be used strictly in accordance with the instructions set out in the Package Insert. IASON will not be held responsible for any loss or damage (except as required by statute) caused, arising out of non-compliance with the instructions provided. CAUTION: this kit contains material of human and/or animal origin. Handle kit reagents as if capable of transmitting an infectious agent.
Source material from Human origin which is used in this kit was tested and found negative for HbsAG and HIV as well as for HCV antibodies. However, since there is no diagnostic procedure that excludes these agents with 100 percent certainty all components should be handled as potentially hazardous material.
Appropriate precautions and good laboratory practices must be used in the storage, handling and disposal of the kit reagents. Disposal of kit reagents should be in accordance with local regulations.

Damaged test kit

In case of serious damage to the test kit or components, the company IASON must be notified in writing at least one week after receiving the kit. Severely damaged single components should not be used for the test run. They have to be stored until a final solution has been found.

Shelf life and storage of reagents

The opened reagents are stable up to the expiry date stated on the label when stored at 2 - 8 °C.

Storage and preparation of serum samples

Use human serum or plasma (citrate, heparin) samples with this assay. If the assay is performed within 5 days after sample collection, the specimen should be kept at 2...8°C; otherwise they should be aliquoted and stored deep-frozen (-70 to -20°C). If samples are stored frozen, mix thawed samples well before testing. Avoid repeated freezing and thawing. Heat inactivation of samples is not recommended.

Sample dilution

Before assaying, all samples should be diluted 1+100 with DIL. Dispense 10µl sample and 1 mL DIL into tubes to obtain a 1+100 dilution and thoroughly mix with a Vortex.

Materials provided

Allow all reagents 1-9 to reach room temperature (20-25°C) before use.

1. MPL Mycoplasma pneumoniae Coated Microplate (IgM): 12 break-apart 8-well snap-off strips coated with Mycoplasma pneumoniae antigens; in resealable aluminium foil.

   The break-apart snap-off strips are coated with Mycoplasma pneumoniae antigens. Immediately after removal of the strips, the remaining strips should be resealed in the aluminium foil along with the desiccant supplied and stored at 2...8 °C.
2. DIL IgM Sample Diluent: 1 bottle containing 100 mL of phosphate buffer (10mM) for sample dilution; pH 7.2 ± 0.2; anti-human IgG (RF Absorbent); coloured green; ready to use; white cap.
3. STOP Stop Solution: 1 bottle containing 15 mL sulphuric acid, 0.2 mol/l; ready to use; red cap.
4. CONJ Mycoplasma pneumoniae anti-IgM Conjugate: 1 bottle containing 20 mL of peroxidase labelled antibody to human IgM in phosphate buffer (10mM); coloured red, ready to use; black cap.
5. SUB TMB Substrate Solution: 1 bottle containing 15 mL 3,3',5,5'-tetramethylbenzidine (TMB);<0.1%; ready to use; yellow cap; <5% NMP.

   The reagent is ready to use and has to be stored at 2...8 °C, away from the light. The solution should be colourless or could have a slight blue tinge. If the substrate turns into blue, it may have become contaminated and should be thrown away.
6. PC Mycoplasma pneumoniae IgM Positive Control: 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; red cap.
7. CUTOFF Mycoplasma pneumoniae IgM Cut-off Control: 1 vial containing 3 mL control (human serum or plasma); coloured yellow; ready to use; green cap.
8. NC Mycoplasma pneumoniae IgM Negative Control: 1 vial containing 2 mL control (human serum or plasma); coloured yellow; ready to use; blue cap.
9. WASH Washing Solution (20x conc.): 1 bottle containing 50 mL of a 20-fold concentrated phosphate buffer (0.2 M), pH 7.2 ± 0.2 for washing the wells; white cap. Dilute washing solution 1+19; e.g. 10 ml washing Buffer + 190 ml distilled water. The diluted buffer is stable for 5 days at room temperature (20-25°C). In case crystals appear in the concentrate, warm up the solution to 37 °C e.g. in a water bath. Mix well before dilution.

Materials required but not provided in the kit

◊ ELISA microwell plate reader, equipped for the measurement of absorbance at 450/620 nm
◊ Incubator 37°C
◊ Manual or automatic equipment for rinsing wells
◊ Pipettes to deliver volumes between 10 and 1000 µl
◊ Vortex tube mixer

- ◊ Distilled water
- ◊ Disposable tubes
- ◊ Timer

Assay procedure

General remarks

1. All reagents and specimens must be allowed to come to room temperature before use. All reagents must be mixed without foaming.
2. Once the test has been started, all steps should be completed without interruption.
3. Use new disposal plastic pipette tips for each standard, control or sample in order to avoid cross contamination
4. Absorbance is a function of the incubation time and temperature. Before starting the assay, it is recommended that all reagents are ready, caps removed, all needed wells secured in holder, etc. This will ensure equal elapsed time for each pipetting step without interruption.
5. As a general rule the enzymatic reaction is linearly proportional to time and temperature.
6. Each assay run must include a standard curve and controls.

Please read the instruction for use carefully before performing the assay. Result reliability depends on strict adherence to the instruction for use as described. The following test procedure is only validated for manual procedure.
If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300 µL to 350 µL to avoid washing effects. Prior to commencing the assay, the distribution and identification plan for all specimens and standards/controls (duplicates recommended) should be carefully established on the plate layout supplied in the kit. Select the required number of MPL or wells and insert them into the holder.

It is recommended to determine controls and patient samples in duplicate, if necessary. Perform all assay steps in the order given and without any appreciable delays between the steps. A clean, disposable tip should be used for dispensing each control and sample. Adjust the incubator to 37° ± 1°C.

1. Dispense 100 µL NC CUTOFF PC and diluted samples into their respective wells. Leave well A1 for substrate blank.
2. Cover wells with the foil supplied in the kit.
3. Incubate for 1 hour ± 5 min at 37±1°C.
4. When incubation has been completed, remove the foil, aspirate the content of the wells and wash each well three times with 300µl of WASH. Avoid overflows from the reaction wells. The interval between washing and aspiration should be >5 sec. At the end carefully remove remaining fluid by tapping strips on tissue paper prior to the next step! Note: Washing is important! Insufficient washing results in poor precision and false results.
5. Dispense 100 µL CONJ into all wells except for the Substrate Blank well (e.g. A1). Cover with foil.

6. Incubate for 30 min at room temperature (20-25°C). Do not expose to direct sunlight.
7. Repeat step 4.
8. Dispense 100 µL SUB into all wells
9. Incubate for exactly 15 min at room temperature (20-25°C) in the dark. A blue colour occurs due to an enzymatic reaction.
10. Dipense 100 µL STOP into all wells in the same order and at the same rate as for the SUB, thereby a colour change from blue to yellow occurs.
11. Measure the absorbance at 450/620 nm within 30 min after addition of the STOP.

or fully automated on:

- **IASON® Quardette**
- **IASON® PersonalLab**
- **IASON® Gladiator**

Measurement

Adjust the ELISA Microwell Plate Reader to zero using the substrate blank in well A1. If - due to technical reasons - the ELISA reader cannot be adjusted to zero using the substrate blank in well A1, subtract the absorbance value of well A1 from all other absorbance values measured in order to obtain reliable results!
Measure the absorbance of all wells at 450 nm and record the absorbance values for each standard/control and patient sample in the plate layout.
Bichrometic measurement using a reference wavelength of 620 nm is recommended.
Where applicable calculate the mean absorbance values of all duplicates.

Results

Run validation criteria

In order for an assay to be considered valid, the following criteria must be met:

Substrate blank	Absorbance value < 0.100.
NC:	Absorbance value < 0.200 and < CUTOFF
CUTOFF:	Absorbance value 0.150 – 1.300.
PC:	Absorbance value > CUTOFF.

If these criteria are not met, the test is not valid and must be repeated.

Calculation of results

The CUTOFF is the mean absorbance value of the CUTOFF determinations.

Example: Absorbance value CUTOFF 0.44 + absorbance value CUTOFF 0.42 =0.86 / 2 = 0.43

CUTOFF = 0.43

Results in IASON arbitrary units (IAU)

$$\frac{\text{Patient (mean) absorbance value} \times 10}{\text{CUTOFF}} = [\text{IASON arbitrary units = IAU}]$$

Example: $\frac{1.591 \times 10}{0.43} = IAU$

Expected values

	IAU
Cut-off	10
Grey zone	9-11
Negative	<9
Positive	>11

Interpretation of results

Cut-off	10 IAU	
Positive	> 11 IAU	Antibodies against the pathogen are present. There has been a contact with the antigen (pathogen resp. vaccine).
Equivocal	9 – 11 IAU	Antibodies against the pathogen could not be detected clearly. It is recommended to repeat the test with a fresh sample in 2 to 4 weeks. If the result is equivocal again the sample is judged as negative.
Negative	< 9 IAU	The sample contains no antibodies against the pathogen. A previous contact with the antigen (pathogen resp. vaccine) is unlikely.
Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.		

Antibody isotypes and state of infection

Serology	Significance
IgM	Characteristic of the primary antibody response High IgM titer with low IgG titer: → suggests a current or very recent infection Rare: → persisting IgM
IgG	Characteristic of the secondary antibody response May persist for several years High IgG titer with low IgM titer: → may indicate a past infection
IgA	Produced in mucosal linings throughout the body (⇒ protective barrier) Usually produced early in the course of the infection

Quality control

The regular use of control samples at several analyte levels is advised to ensure day-to-day validity of results. Controls should be tested as unknowns. Quality Control charts should be maintained to follow the assay performance.
Good laboratory practice requires that controls be run with each calibration curve. A statistically significant number of controls should be assayed to establish mean values and acceptable ranges to assure proper performance.
It is recommended to use control samples according to state and federal regulations. The use of control samples is advised to assure the day to day validity of results. Use controls at both normal and pathological levels.
The controls and the corresponding results of the QC-Laboratory are stated in the QC certificate added to the kit. The values and ranges stated on the QC sheet always refer to the current kit lot and should be used for direct comparison of the results.
It is also recommended to make use of national or international Quality Assessment programs in order to ensure the accuracy of the results.
Employ appropriate statistical methods for analysing control values and trends. If the results of the assay do not fit to the established acceptable ranges of control materials patient results should be considered invalid. In this case, please check the following technical areas: Pipetting and timing devices; photometer, expiration dates of reagents, storage and incubation conditions, aspiration and washing methods.
After checking the above mentioned items without finding any error contact your distributor or IASON GmbH directly.

Test characteristics

Precision

Intra assay cv (n = 24)			Inter assay cv n =12		
Sample	**Mean OD**	**CV [%]**	**Sample**	**Mean OD**	**CV [%]**
A	0.526	5.02	1	19.56	7.08
B	0.905	6.20	2	18.16	13.13
C	1.074	6.34	3	5.28	7.31

Diagnostic specificity

The diagnostic specificity is defined as the probability of the assay of scoring negative in the absence of the specific analyte. It is 99.29% (95 % confidence interval: 96.11%-99.98%).

Diagnostic sensitivity

The diagnostic sensitivity is defined as the probability of the assay of scoring positive in the presence of the specific analyte. It is 100 % (95% confidence interval: 95.01%-100%).

Interferences

Interferences with hemolytic, lipemic or icteric samples are not observed up to a concentration of 10 mg/mL hemoglobin, 5 mg/mL triglycerides and 0.5 mg/mL bilirubin.

Cross Reactivity

Investigation of a sample panel with antibody activities to potentially cross-reacting parameters did not reveal evidence of false-positive results due to cross-reactions.

Limitation of the procedure

Bacterial contamination or repeated freeze-thaw cycles of the specimen may affect the absorbance values. Diagnosis of an infectious disease should not be established on the basis of a single test result. A precise diagnosis should take into consideration clinical history, symptomatology as well as serological data. In immunocompromised patients and newborns serological data only have restricted value.

Legal aspects

Reliability of results

The test must be performed exactly as per the manufacturer's instructions for use. Moreover the user must strictly adhere to the rules of GLP (Good Laboratory Practice) or other applicable national standards and/or laws. This is especially relevant for the use of control reagents. It is important to always include, within the test procedure, a sufficient number of controls for validating the accuracy and precision of the test.
The test results are valid only if all controls are within the specified ranges and if all other test parameters are also within the given assay specifications. In case of any doubt or concern please contact IASON.

Therapeutic consequences

Therapeutic consequences should never be based on laboratory results alone even if all test results are in agreement with the items as stated under Reliability of Results. Any laboratory result is only a part of the total clinical picture of a patient.
Only in cases where the laboratory results are in acceptable agreement with the overall clinical picture of the patient should therapeutic consequences be derived.
The test result itself should never be the sole determinant for deriving any therapeutic consequences.

Liability

Any modification of the test kit and/or exchange or mixture of any components of different lots from one test kit to another could negatively affect the intended results and validity of the overall test. Such modification and/or exchanges invalidate any claim for replacement.
Claims submitted due to customer misinterpretation of laboratory results subject to point Therapeutic Consequences are also invalid. Regardless, in the event of any

claim, the manufacturer's liability is not to exceed the value of the test kit. Any damage caused to the test kit during transportation is not subject to the liability of the manufacturer.

Useful publications

◊ Mycoplasmataceae (2009) In Herbert Hof, Rüdiger Dörries, Gernot Geginat: Medizinische Mikrobiologie; 237 Tabellen 4., vollst. überarb. und erw. Aufl. Stuttgart: Thieme (Duale Reih), pp. 452-455.

◊ Clyde, Wallace A., JR (1993): Clinical overview of typical Mycoplasma pneumoniae infections. In Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 17 Suppl 1, S32-6.

◊ Foy, Hjordis M. (1993): Infections caused by Mycoplasma pneumoniae and possible carrier state in different populations of patients. In Clinical infectious diseases : an official publication of the Infectious Diseases Society of America 17 Suppl 1, S37-46.

◊ Jacobs, E. (1993): Serological diagnosis of Mycoplasma pneumoniae infections: a critical review of current procedures. In Clinical infectious diseases: an official publication of the Infectious Diseases Society of America 17 Suppl 1, S79-82.

◊ Kayser, Fritz H. (2005): Bacteria as Human Pathogens. In Fritz H. Kayser, Kurt A. Bienz, Johannes Eckert, Rolf M. Zinkernagel: Medical microbiology. Stuttgart, New York: Thieme (Thieme Flexibook), pp. 229–346.

◊ O'Handley, John G.; Gray, Larry D. (1997): The incidence of Mycoplasma pneumoniae pneumonia. In The Journal of the American Board of Family Practice 10 (6), pp. 425–429.

◊ Vikerfors, Tomas; Brodin, Glenn; Grandien, Monica; Hirschberg, Lotta; Krook, Aud; Pettersson, Carl-Axel (1988): Detection of specific IgM antibodies for the diagnosis of Mycoplasma pneumoniae infections: a clinical evaluation. In Scandinavian journal of infectious diseases 20 (6), pp. 601–610.

◊ Wiegand, R. (1994): Mycoplasma pneumoniae. In Henning Brandis, Hans J. Eggers, W. Köhler, Gerhard Pulverer (Eds.): Lehrbuch der medizinischen Mikrobiologie. 7. Aufl. Stuttgart: Fischer; G. Fischer.

Pipetting scheme

Allow all reagents to reach room temperature.
Dilute samples 1 + 100 with DIL

Step	Procedure
1. Pipetting	NC PC CUTOFF diluted samples **100 µL** Leave well A1 for substrate blank Cover wells with foil supplied in the kit
2. Incubation	1 hour at 37°C
3. Washing	Aspirate or decant, add 300* µL WASH repeat wash step two times and dry on absorbent material
4. Pipetting	CONJ **100 µL** except blank
5. Incubation	30 min at room temperature (20-25°C)
6. Washing	Aspirate or decant, pipetting 300* µL WASH repeat wash step two times and dry on absorbent material
7. Pipetting	SUB **100 µL**
8. Incubation	15 min at room temperature (20-25°C) in the dark
9. Pipetting	STOP **100 µL**
10. Reading	450nm (RF 620nm) Overrange Filter: 405nm, Faktor 3 (depends on photometer), reading within 30 min
11. Calculation	Patient (mean) absorbance value x 10/CUTOFF

* If performing the test on ELISA automatic systems we recommend to increase the washing steps from three to five and the volume of washing solution from 300µl to 350µl to avoid washing effects.

Expected values

	IAU
Cut-off	10
Grey zone	9-11
Negative	<9
Positive	>11