Mycoplasma pneumoniae IgG ELISA

NB-06-0207

Contents **Page**

Mycoplasma pneumoniae IgG ELISA

#Cat : NB-06-0207 Size : 1x96 Tests

1. Intended Use

The Neo Biotech Mycoplasma pneumoniae IgG antibody ELISA kit has been designed for the detection and the quantitative determination of specific IgG antibodies against Mycoplasma pneumoniae in serum and plasma. Further applications in other body fluids are possible and can be requested from the Technical Service of Neo Biotech. This assay is intended for research use only.

2. General Information

Mycoplasms belong to the Mollicutes class. Common characteristics of the six eubacterial genera is the lack of a bacterial cell wall, osmotic fragility and small dimensions, which allow a penetration through a 0.45 µm filter. Also the genome with 600 kbp is significantly smaller compared with gram-positive and gram-negative bacteria. Out of this reason they have never been found as freely living organisms. In nature Mollicutes depend on a host cell, respectively, on a host organism like a parasite.

Mycoplasma pneumoniae is a human pathogenic bacterium causing tracheobronchitis and primary atypical pneumonia. Associated with the host cell, surface colonization of human respiratory tract epithelial cells takes place. Also secondary diseases like infarction, encephalitis, chronic neuropathy and the Guillain-Barre syndrome can in some cases be connected with a M. pneumoniae infection.

In the laboratory, M. pneumoniae can be grown without a host cell in rich medium supplemented with 10-20% horse serum. Besides the cold agglutinin test and complement fixation reaction CF, ELISA is the method of choice, which shows an excellent sensitivity and the possibility to differentiate between the immunogloblin classes.

Specific IgA antibodies were developed more regularly and more rapidly than IgM during an acute infection. IgA titres also started to decrease earlier than IgM or the late-peaking IgG response.

It could be shown in various studies, that the determination of all the three immunoglobulin classes is necessary, to monitor each step of the course.

3. Principle of the Test

The Neo Biotech Mycoplasma pneumoniae IgG antibody test kit is based on the principle of the enzyme immunoassay (EIA). Mycoplasma pneumoniae antigen is bound on the surface of the microtiter strips. Diluted sample serum or ready-to-use standards are pipetted into the wells of the microtiter plate. A binding between the IgG antibodies of the serum and the immobilized Mycoplasma pneumoniae antigen takes place. After a one hour incubation at room temperature, the plate is rinsed with diluted wash solution, in order to remove unbound material. Then ready-to-use anti-human-IgG peroxidase conjugate is added and incubated for 30 minutes. After a further washing step, the substrate (TMB) solution is pipetted and incubated for 20 minutes, inducing the development of a blue dye in the wells. The color development is terminated by the addition of a stop solution, which changes the color from blue to yellow. The resulting dye is measured spectrophotometrically at the wavelength of 450 nm. The concentration of IgG antibodies is directly proportional to the intensity of the color.

4. Limitations, Precautions and General Comments

- Only for research use! Do not ingest or swallow! The usual laboratory safety precautions as well as the prohibition of eating, drinking and smoking in the lab have to be followed.
- All sera and plasma or buffers based upon, have been tested respective to HBsAg, HIV and HCV with recognized methods and were found negative. Nevertheless precautions like the use of latex gloves have to be taken.
- Serum and reagent spills have to be wiped off with a disinfecting solution (e.g. sodium hypochlorite, 5%) and have to be disposed of properly.
- All reagents have to be brought to room temperature (18 to 25 °C) before performing the test.
- Before pipetting all reagents should be mixed thoroughly by gentle tilting or swinging. Vigorous shaking with formation of foam should be avoided.
- It is important to pipet with constant intervals, so that all the wells of the microtiter plate have the same conditions.
- When removing reagents out of the bottles, care has to be taken that the stoppers are not contaminated. Further a possible mix-up has to be avoided. The content of the bottles is usually sensitive to oxidation, so that they should be opened only for a short time.
- In order to avoid a carry-over or a cross-contamination, separate disposable pipet tips have to be used.
- No reagents from different kit lots have to be used, they should not be mixed among one another.
- All reagents have to be used within the expiry period.
- In accordance with a Good Laboratory Practice (GLP) or following ISO9001 all laboratory devices employed should be regularly checked regarding the accuracy and precision. This refers amongst others to microliter pipets and washing or reading (ELISA-Reader) instrumentation.
- The contact of certain reagents, above all the stopping solution and the substrate with skin, eye and mucosa has to be avoided, because possible irritations and acid burns could arise, and there exists a danger of intoxication.

5. Reagents Provided

Store kit components at 2-8°C and do not use after the expiry date on the box outer label. Before use, all components should be allowed to warm up to ambient temperature (18-25°C). After use, the plate should be resealed, the bottle caps replaced and tightened and the kit stored at 2-8°C. The opened kit should be used within three months.

Components	Volume / Qty.
Mycoplasma pneumoniae antigen coated microtiter strips	12
Calibrator A (Negative Control)	2 mL
Calibrator B (Cut-Off Standard)	2 mL
Calibrator C (Weak Positive Control)	2 mL
Calibrator D (Positive Control)	2 mL
Enzyme Conjugate	15 mL
Substrate	15 mL
Stop Solution	15 mL
Sample Diluent	60 mL
Washing Buffer (10×)	60 mL
Plastic foils	2
Plastic bag	1

5.1. Microtiter Strips

12 strips with 8 breakable wells each, coated with a Mycoplasma pneumoniae antigen (purified complete antigen, strain FH, with P1-adhesin addition). Ready-to-use.

5.2. Calibrator A (Negative Control)

2 mL, protein solution diluted with PBS, contains no IgG antibodies against Mycoplasma pneumoniae. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-touse.

5.3. Calibrator B (Cut-Off Standard)

2 mL human serum diluted with PBS, contains a low concentration of IgG antibodies against Mycoplasma pneumoniae. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.4. Calibrator C (Weak Positive Control)

2 mL, human serum diluted with PBS, contains a medium concentration of IgG antibodies against Mycoplasma pneumoniae. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.5. Calibrator D (Positive Control)

2 mL, human serum diluted with PBS, contains a high concentration of IgG antibodies against Mycoplasma pneumoniae. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane. Ready-to-use.

5.6. Enzyme Conjugate

15 mL, anti-human-IgG-HRP (rabbit), in protein-containing buffer solution. Addition of 0.01 % methylisothiazolone and 0.01 % bromonitrodioxane and 5 mg/L Proclin™. Ready-to-use.

5.7. Substrate

15 mL, TMB (tetramethylbenzidine). Ready-to-use.

5.8. Stop Solution

15 mL, 0.5 M sulfuric acid. Ready-to-use.

5.9. Sample Diluent
60 mL, PBS/BSA buffer. Addition of 0.095 % sodium azide. Ready-to-use.

5.10. Washing Buffer
60 mL, PBS + Tween 20, 10x concentrate. Final concentration: dilute 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

5.11. Plastic Foils
2 pieces to cover the microtiter strips during the incubation.

5.12. Plastic Bag
Resealable, for the dry storage of non-used strips.

6. Materials Required but not Provided

- 5 µL-, 100 µL- and 500 µL micro- and multichannel pipets
- Microtiter Plate Reader (450 nm)
- Microtiter Plate Washer
- Reagent tubes for the serum dilution
- Bidistilled water

7. Specimen Collection and Handling

Principally serum or plasma (EDTA, heparin) can be used for the determination. Serum is separated from the blood, which is aseptically drawn by venipuncture, after clotting and centrifugation. The serum or plasma samples can be stored refrigerated (2-8°C) for up to 48 hours, for a longer storage they should be kept at -20 °C. The samples should not be frozen and thawed repeatedly. Lipemic, hemolytic or bacterially contaminated samples can cause false positive or false negative results.

For the performance of the test the samples (not the standards) have to be diluted 1:101 with readyto-use sample diluent (e.g. 5 µL serum + 500 µL sample diluent).

8. Assay Procedure

8.1. Preparation of Reagents

Washing Solution: dilute before use 1+9 with distilled water. If during the cold storage crystals precipitate, the concentrate should be warmed up at 37°C for 15 minutes.

- Strict adherence to the protocol is advised for reliable performance. Any changes or modifications are the responsibility of the user.
- All reagents and samples must be brought to room temperature before use, but should not be left at this temperature longer than necessary.
- Standards and samples should be assayed in duplicates.
- A standard curve should be established with each assay.
- Return the unused microtiter strips to the plastic bag and store them dry at 2-8°C.

8.2. Assay Steps

1. Prepare a sufficient amount of microtiter wells for the standards, controls and samples in duplicate as well as for a substrate blank.
2. Pipet 100 µL each of the **diluted** (1:101) samples and the **ready-to-use** standards and controls respectively into the wells. Leave one well empty for the substrate blank.
3. Cover plate with the enclosed foil and incubate at room temperature for 60 minutes.

4. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
5. Pipet 100 µL each of ready-to-use conjugate into the wells. Leave one well empty for the substrate blank.
6. Cover plate with the enclosed foil and incubate at room temperature for 30 minutes.
7. Empty the wells of the plate (dump or aspirate) and add 300 µL of diluted washing solution. This procedure is repeated totally three times. Rests of the washing buffer are afterwards removed by gentle tapping of the microtiter plate on a tissue cloth.
8. Pipet 100 µL each of the ready-to-use substrate into the wells. This time also the substrate blank is pipetted.
9. Cover plate with the enclosed foil and incubate at room temperature for 20 minutes in the dark (e.g. drawer).
10. To terminate the substrate reaction, pipet 100 µL each of the ready-to-use stop solution into the wells. Pipet also the substrate blank.
11. After thorough mixing and wiping the bottom of the plate, perform the reading of the absorption at 450 nm (optionally reference wavelength of 620 nm). The color is stable for at least 60 minutes.

9. Evaluation

The mean values for the measured absorptions are calculated after subtraction of the substrate blank value. The difference between the single values should not exceed 10 %.

Example

	OD Value	corrected OD	Mean OD Value
Substrate Blank	0.020		
Negative Control	0.077 / 0.059	0.057 / 0.039	0.048
Cut-Off Standard	0.537 / 0.501	0.517 / 0.481	0.499
Weak Positive Control	1.069 / 1.103	1.049 / 1.083	1.066
Positive Control	2.041 / 2.069	2.021 / 2.049	2.035

The above table contains only an example, which was achieved under arbitrary temperature and environmental conditions. The described data constitute consequently **no reference values** which have to be found in other laboratories in the same way.

9.1. Qualitative Evaluation

The calculated absorptions for the sample sera, as mentioned above, are compared with the value for the cut-off standard. If the value of the sample is higher, there is a positive result.

For a value below the cut-off standard, there is a negative result. It seems reasonable to define a range of +/-20 % around the value of the cut-off as a grey zone. In such a case the repetition of the test with the same serum or with a new sample of the same individual, taken after 2-4 weeks, is recommended. Both samples should be measured in parallel in the same run.

The positive control must show at least the double absorption compared with the cut-off standard.

9.2. Quantitative Evaluation

The ready-to-use standards and controls of the Mycoplasma antibody kit are defined and expressed in arbitrary units (U/mL). This results in an exact and reproducible quantitative evaluation. Consequently for a given sample follow-up controls become possible. The values for controls and standards in units are printed on the labels of the vials.

For a quantitative evaluation the absorptions of the standards and controls are graphically drawn against their concentrations. From the resulting reference curve the concentration values for each sample can then be extracted in relation to their absorptions. It is also possible to use automatic computer programs.

10. Assay Characteristics

Mycoplasma ELISA	IgG	IgA	IgM
Intra-AssayPrecision	7.9 %	10.7 %	7.9 %
Inter-AssayPrecision	7.7 %	9.2 %	8.0 %
Inter-Lot-Precision	4.2 – 10.2 %	5.4 – 9.3 %	5.1 – 12.5 %
Analytical Sensitivity	1.12 U/mL	1.10 U/ml	1.22 U/mL
Recovery	85 – 100 %	79 – 89 %	91 – 116 %
Linearity	82 – 128 %	97 – 135 %	70 – 106 %
Cross-Reactivity	No cross-reactivity to RSV, Influenza, Parainfluenza, Adenovirus.		
Interferences	No interferences to bilirubin up to 0.3 mg/mL, hemoglobin up to 8.0 mg/mL and triglycerides up to 5.0 mg/mL		
Clinical Specificity	80 %	99 %	92 %
Clinical Sensitivity	96 %	100 %	100 %

11. References

1. Brunner H et al. Determination of IgG, IgM, and IgA antibodies to Mycoplasma pneumoniae by an indirect staphylococcal radioimmunoassay. Med. Microbiol. Immunol, **165**: 29 (1978).
2. Chia WK et al. Development of urease conjugated enzyme-linked immunosorbent assays (ELISA) for the detection of IgM and IgG antibodies against Mycoplasma pneumoniae in human sera. Diagn. Microbiol. Infect. Dis., **11**: 101 (1988).
3. Elfaki M.G. An enzyme-linked immunosorbent assay for the detection of specific IgG antibody to Mycoplasma gallisepticum in sera and tracheobronchial washes. J. Immunoassay, **13**: 97 (1992).
4. Gogate A et al. Detection of Chlamydia trachomatis antigen & Toxoplasma gondii (IgM) & Mycoplasma hominis (IgG) antibodies by ELISA in women with bad obstetric history. Indian J. Med. Res., **100**: 19 (1994).
5. Granstrom M et al. The role of IgA determination by ELISA in the early serodiagnosis of Mycoplasma pneumoniae infection, in relation to IgG and mu-capture IgM methods. J. Med. Microbiol., **40**: 288 (1994).
6. Gurfinkel EP et al. IgG antibodies to chlamydial and mycoplasma infection plus C-reactive protein related to poor outcome in unstable angina. Arch. Inst. Cardiol. Mex., **67**: 462 (1997).
7. Hirschberg L et al. IgG response to Mycoplasma pneumoniae in patients with communityacquired pneumonia determined by ELISA. APMIS, **96**: 605 (1988).
8. Seggev JS. Isotype-specific antibody responses to acute Mycoplasma pneumoniae infection. Ann. Allergy Asthma Immunol., **77**: 67 (1996).
9. Smith TF. Mycoplasma pneumoniae infections: diagnosis based on immunofluorescence titer of IgG and IgM antibodies. Mayo Clin. Proc., **61**: 830 (1986).
10. Uldum SA et al. Enzyme immunoassay for detection of immunoglobulin M (IgM) and IgG antibodies to Mycoplasma pneumoniae. J. Clin. Microbiol., **30**: 1198 (1992).

For research use only.