Mycoplasma pneumoniae IgA ELISA Kit

Catalog Number KA1463

96 assays

Version: 02

Intended for research use only

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Introduction

Intended Use

The *Mycoplasma pneumoniae* IgA ELISA Kit is for detection of IgA antibodies to *Mycoplasma pneumoniae.*

Background

The kit is intended for research of diseases caused by bacterium *Mycoplasma pneumoniae*. This test is used in research of all inflammations in the respiratory system - from mild catarrh to severe pneumonia and their complications such as pericarditis, meningoencephalitis, otitis and erythema nodosum.
It is recommended to monitor the dynamics of antibodies levels to improve the detecting value of the test. In such case, two serum samples are withdrawn from the same person. The first sample is taken during the acute phase of the disease and the second is withdrawn ten to fifteen days later - at which time the changes of the particular antibodies level are expected. Considering the antibody isotype switching during infection, it is recommended to test all antibody isotypes (lgG, lgA and IgM) simultaneously in each sample.

Principle of the Assay

The *Mycoplasma pneumoniae* IgA ELISA Kit is a solid-phase immunoanalytical test. The strips are coated with specific antigen. The anti-*Mycoplasma pneumoniae* antibodies, if present in the tested sera, bind to the immobilized antigens and the antibodies being in complexes with antigen are later on recognised by animal anti-human lgA antibodies labelled with horseradish peroxidase. The labelled antibodies are revealed by an enzymatic reaction with a chromogenic substrate. Negative sera do not react – a mild change in colour, if present, may be attributed to the reaction background.

Flow Chart

Step 1.	Prepare reagents and samples
	↓
Step 2.	Dispense 100 µL/well of controls and samples
	↓
	Incubate for 30 minutes at 37˚C
	↓
	Aspirate and Wash 4 times (250 µL/well)
	↓
Step 3.	Dispense 100 µL/well of Px-conjugate r.t.u.
	↓
	Incubate 30 minutes at 37˚C
	↓
	Aspirate and Wash 4 times (250 µL/well)
	↓
Step 4.	Dispense 100 µL/well of TMB substrate
	↓
	Incubate 15 minutes in dark at room temperature
	↓
Step 5.	Dispense 100 µL/well of Stop solution
	↓
Step 6.	Read the absorbance at 450/620-690 nm within 20 minutes

General Information

Materials Supplied

List of component

Component	Amount
ELISA break-away strips coated with specific antigen	1 microplate
Calibrator r.t.u.[1)]	1.3 mL
Negative control serum r.t.u.	1.3 mL
Positive control serum r.t.u.	1.3 mL
Anti-human IgA antibodies labelled with horseraddish peroxidase (Px-conjugate) r.t.u.	13 mL
Wash buffer 10x concentrated anti-Mycoplasma pneum.	55 mL
Dilution buffer anti-Mycoplasma pneum. r.t.u.	60 mL
Chromogenic substrate (TMB substrate) r.t.u.	13 mL
Stop solution r.t.u.	13 mL

[1)] r.t.u. ready to use

Note: Control sera may be colorless to yellowish or blue due to the use of different diluents.

Storage Instruction

The ELISA kit should be used within three months after opening.

Store the kit and the kit reagents at +2 to +10°C, in a dry place and protected from the light. Store unused strips in the scalable pouch and keep the desiccant inside.

If you find damage at any kit component, please inform the manufacturer.

Expiration date is indicated at the ELISA kit label and at all reagent labels.

Materials Required but Not Supplied

- ✓ Distilled or deionised water for dilution of Wash buffer concentrate.
- ✓ Appropriate equipment for pipetting, liquid dispensing and washing.
- ✓ Spectrophotometer/colorimeter (microplate reader — wavelenght 450 nm). Thermostat (set at 37°C) for ELISA plate incubation.

Precautions for Use

- ✓ Safety Precautions
- All ingredients of the kit are for laboratory use only.
- Controls contain human sera that has been tested negative for HBsAg, anti-HIV-1,2 and anti-HCV. However they should be regarded as contagious and handled and disposed of according to the

appropriate regulations.

- Autoclave all reusable materials that were in contact with human samples for 1 hour at 121°C, bum disposable ignitable materials, decontaminate liquid wastes and nonignitable materials with 3% chloramine.
- Liquid wastes containing acid (Stop solution) should be neutralized in 4% sodium bicarbonate solution.
- Handle Stop solution with care. Avoid contact with skin or mucous membranes. In case of contact with skin, rinse immediately with plenty of water and seek medical advice.
- The waste from strip washing disinfect in waste container using appropriate disinfection solution (e.g.Incidur, Incidin, chloramin, ...) in concentrations recommended by the producer.
- Do not smoke, eat or drink during work. Do not pipette by mouth. Wear disposable gloves while handling reagents or samples and wash your hands thoroughly afterwards. Avoid spilling or producing aerosol.

✓ Handling Precautions

- Manufacturer guarantees performance of the entire ELISA kit.
- Follow the assay procedure indicated in the protocol.
- Calibrator and control sera contain preservative ProClin 300® (mix of 5-Chloro-2-methyl-4-isothiazolin-3-one and 2-Mcthyl-2H-isothiazol-3-one (3:1)).
- Avoid microbial contamination of serum samples and kit reagents.
- Avoid cross-contamination of reagents.
- Avoid contact of the TMB substrate with oxidizing agents or metal surfaces.
- Variations in the test are usually due to:

  * Insufficient mixing of reagents and samples
  * Inaccurate pipetting and inadequate incubation times
  * Poor washing technique or spilling the rim of well with sample or Px-conjugate
  * Use of identical pipette tip for different solutions

Assay Protocol

Reagent Preparation

- ✓ Allow all kit components to reach room temperature. Turn on the thermostat to 37°C.
- ✓ Vortex samples, Calibrator, the Controls and Px-conjugate in order to ensure homogeneity and mix all solutions well prior use.
- ✓ Prepare Wash buffer by diluting the Wash buffer concentrate 10 times with an appropriate volume of distilled or deionised water (e.g. 50 mL of the concentrated Wash buffer + 450 mL of distilled water). If there are crystals of salt present in the concentrated Wash buffer, warm up the vial to +32 to +37°C in a water bath. Diluted Wash buffer is stable for one month at laboratory temperature.
- ✓ Do not dilute Px-conjugate, TMB substrate and Stop solution, they are ready to use.
- ✓ Do not dilute the Control sera and Calibrator, they are ready to use.

Sample Preparation

- ✓ Dilute serum samples 101x with Dilution buffer and mix (e.g. 5 µL of serum sample + 500 µL of Dilution buffer).
- ✓ Store serum samples at +2 to +10°C up to one week. For long period make aliquots and keep them at -18°C- -28°C. Avoid repeated thawing and freezing.
- ✓ Do not store diluted samples. Always prepare fresh.

Assay Procedure

Manufacturer will not be held responsible for results if manual is not followed exactly.

1. Allow the vacuum-closed aluminium bag with strips to reach a room temperature. Withdraw the adequate number of strips and put unused strips into the provided pouch and seal it carefully with the desiccant kept inside.
2. Pipette 100 µL of Dilution buffer, Controls and serum samples to the wells according to the pipetting scheme in Plate Layout: start with filling the first well with Dilution buffer to estimate reaction background the next two wells with Calibrator, next one well with Positive control serum and the following one well with Negative control serum. Fill the remaining wells with diluted serum samples (S1, S2, S3...). It is satisfactory to apply samples as singles, however, if you want to minimize a laboratory error then apply the Controls and samples in doublets and Calibrator in triplet.
   Incubate 30 minutes (± 2 min) at 37°C.
3. Aspirate the liquid from the wells into a collecting bottle containing appropriate disinfectant (see Safety Precautions). Wash and aspirate the wells four times with 250 µL/well of Wash buffer. Avoid cross-contamination between wells!
   If some liquid remains in the wells, invert the plate and tap it on an adsorbent paper to remove the last

remaining drops.

4. Mix Px-conjugate r.t.u. and add 100 µL of Px-conjugate into each well.
   Incubate 30 minutes (± 2 min) at 37°C.
5. Aspiratc and wash four times with 250 µL/well of Wash buffer.
6. Dispense 100 µL of TMB substrate into each well. Incubate for 15 minutes (± 30 seconds) at room temperature.
   The time measurement must be started at the beginning of TMB dispensing. Cover the strips with an aluminium foil or keep them in the dark during the incubation with TMB substrate.
7. Stop the reaction by adding 100 µL of Stop solution. Use the same pipetting rhythm as with the TMB substrate to ensure the same reaction time in all wells. Tap gently the microplate for a few times to ensure complete mixing of the reagents.
8. Read the absorbance at 450 nm with a microplate reader within 20 minutes. It is recommended to use reference reading at 620-690 nm.

Data Analysis

Calculation of Results

First, subtract the absorbance of the background (absorbance of the Dilution buffer well) from the absorbancies of all other wells.

If the absorbance of controls or tested sera are negative after background subtraction, consider them as zero value.

- Qualitative evaluation

1. Compute the mean absorbance of the parallels of Calibrator. If you use three parallels and any value is different from the mean more than 20 % then exclude the deviating well from the calculation and compute a new mean using the remaining two wells.
2. Compute the cut-off value by multiplying the Calibrator mean with a Correction factor. The Correction factor value for the Calibrator determined for this lot of the kit is stated in the Quality control certificate.
3. Assign the samples with absorbances less than 90% of cut-off value as negative and the samples with absorbances higher than 110% of cut-off value as positive.

- Semiquantitative evaluation

Determination of sample Positivity Index:

1. Compute the cut-off value (see the previous paragraph)
2. Compute the Positivity Index for each sample according to the following formula:

$$\text{Sample Positivity Index} = \frac{\text{Sample absorbance}}{\text{Cut-off value}}$$

3. Express serum reactivity in accordance with data in Table 1 (Evaluation of results)

Table 1 Evaluation of results

Index value	Interpretation
< 0.90	Negative
0.90- 1.10	+/-
> 1.10	Positive*

*On the basis of the Positivity Index Value it is possible to estimate semiquantitatively the amount of antibodies in the sample.

Note! Indifferent sample reactivity, i.e. interpreted as +/-, requires repetition of the sample testing. If the result is repetitively indifferent then it is recommended to use an alternative testing method or to obtain another sample from the individual withdrawn 1-2 weeks later.

Example of calculation:

Calibrator OD value = 1.446; 1.354; 1.525

Mean Calibrator OD value = 1.442

OD of tested sera = 0.800

Correction factor = 0.17

Cut-off value = 1.442*0.17 = 0.245

Index value = 0.800/0.245 = 3.27

✓ Interpretation of Results

During result interpretation, it is suitable to determine all 3 isotypes of antibodies (IgG, lgM, lgA) in two samples from one person (first in acute phase and second 10-15 days later).

IgG	lgM	IgA	Result interpretation
-	-		Seronegativity (without contact with *Mycoplasma pneumoniae*)
-	+	+	Early phase of acute infection
-	-	+	Early acute infection without appearance IgM (or solitary persistence of IgA)
+	+	+	Late acute phase of infection
+	(+)	(+)	Post-acute phase of infection
+	-	(+)	Anamnestic antibodies (infection in person‘s history)
+	-	(+)	Reinfection

Performance Characteristics

- Validity of the test

The test is valid if:

The background absorbance (the absorbance of the Dilution buffer) is less than 0.150.

The mean absorbance values of standards (control sera), and the ratio between the absorbance values of Positive control serum/Calibrator are in the ranges stated in the Quality control certificate for this kit lot.

- Precision of the test

The intraassay variability (within the test) and the interassay variability (between tests) evaluation was performed with samples of variable absorbance values.

✓ Intraassay variability example

The coefficient of intraassay variability is max. 8%. It is measured for each particular Lot at least on 12 parallels of the same microtitration plate.

Example: (N = number of parallels of the same microtitration plate, SD = standard deviation)

n	A	± σ	CV
16	1.355	0.050	3.8%
16	0.614	0.023	3.7%

✓ Interassay variability example

The coefficient of interassay variability is max. 15%. It is measured for each particular Lot as comparison of the OD values of the same serum sample in several consecutive tests.

Example: (N = number of parallels of the same microtitration plate, SD = standard deviation)

n	A	± σ	min-max	CV
18	1.369	0.064	1.223-1.476	4.7%
18	0.463	0.060	0.337-0.569	12.9%

- ✓ Recovery test

  Measured values of recovery test for every Lot between 80-120% of expected values.

Resources

References

1. M Karppclin, K 1-lakkarainen, M Kleemola, and A Miettinen: Comparison of three serological methods for diagnosing Mycoplasma pneumoniae infection. Clin Pathol.; 46(12): H20-1123, December I993.
2. M. Granstrom, T. Holme, A. M. Sjogren, A. Ortqvist and M. Kalin: The role of lgA determination by ELISA in the early serodiagnosis of Mycoplasma pneumoniae infection, in relation to lgG and mu-capture lgM methods. The Journal of Medical Microbiology, Vol 40, Issue 4 288-292, 1994.
3. Ken B. Waites and Deborah F. Talkington: Mycoplasma pneumoniae and lts Role as Human Pathogen. Clinical Microbiology Reviews, p. 697-728, Vol. I7, No. 4, October 2004.

Plate Layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	Dilution buffer	Sample 4										
B	Calibrator	Sample…										
C	Calibrator											
D	Control+											
E	Control-											
F	Sample 1											
G	Sample 2											
H	Sample 3											