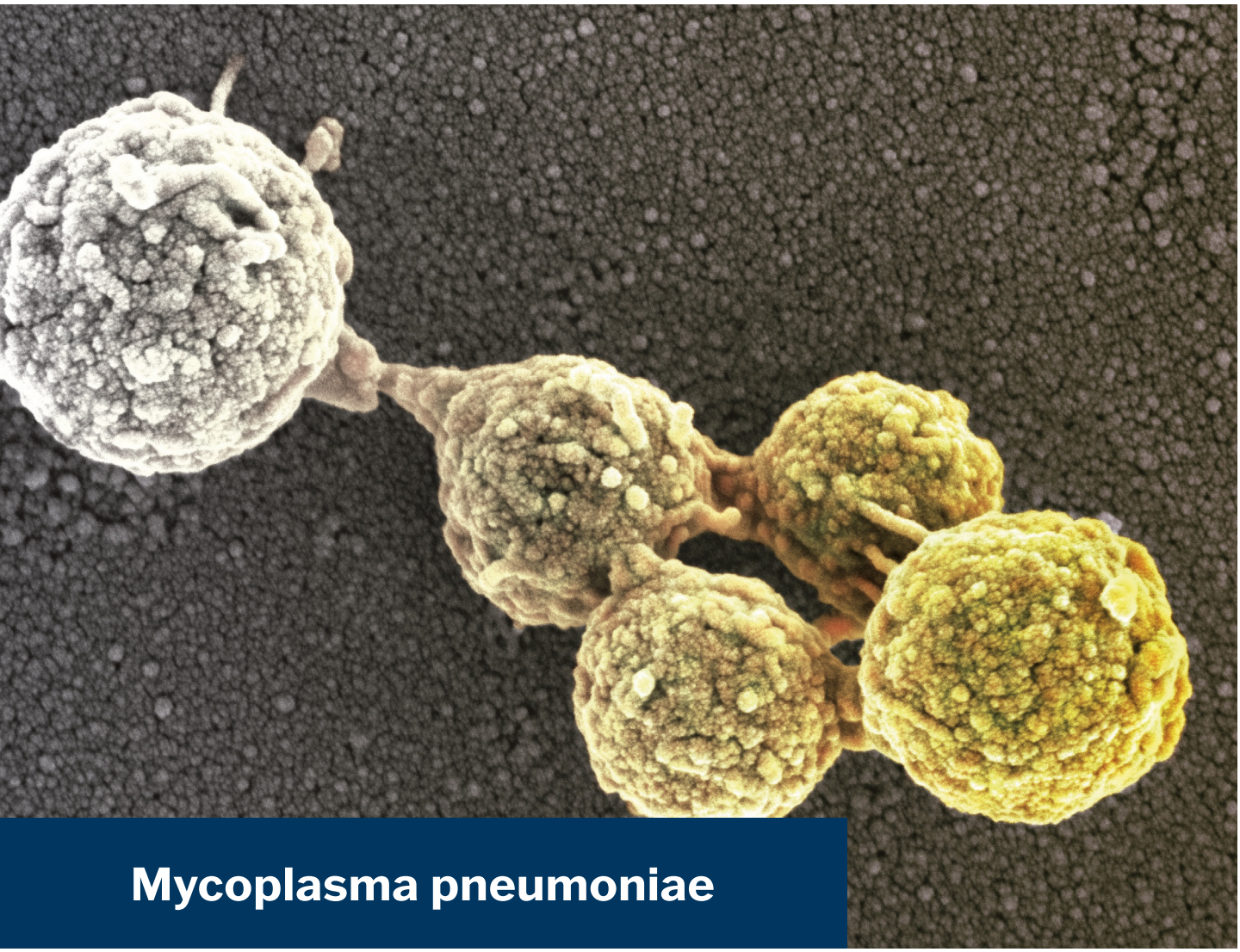


Mycoplasma pneumoniae

Enzyme immunoassays for the diagnostics of Mycoplasma infection

ELISA, **IMUNOBLOT**, and **MICROBLOT-ARRAY** kits are optimized and validated for detection of IgA, IgG, and IgM antibodies in human serum and plasma

CE IVD

Diagnostic kits are intended for professional use in the laboratory.

BG TestLine®

Introduction

Mycoplasma pneumoniae is a primary pathogenic agent of the human respiratory tract. It causes pneumonia accompanied by fever, nausea, ague, cough and fatigue. The disease is prolonged but well curable with antibiotics. The pathogen is airborne, spread especially in dense gatherings of children, particularly during spring and autumn months.

Diagnosis of Infection

Diagnosis of the disease is based on the overall clinical picture, epidemiological anamnesis and laboratory tests. Because it is difficult to cultivate *Mycoplasma pneumoniae*, it is advisable to use the ELISA method for the detection of specific antibodies in human serum or plasma in routine laboratory practice.

Diagnostic Importance of Antibody Classes

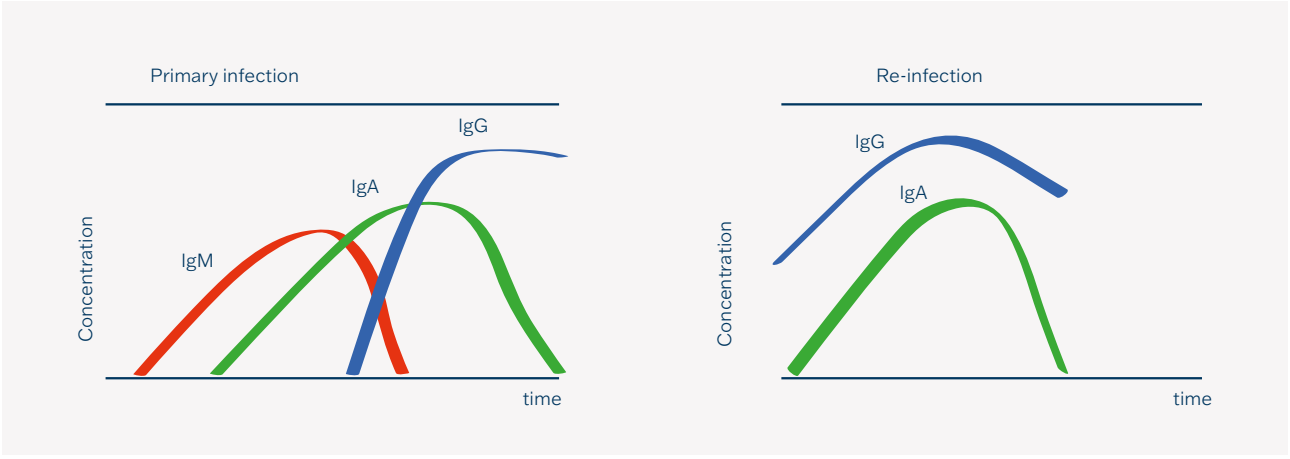
IgM: Primary infection is indicated by IgM antibody increase (1-2 weeks after infection), which reaches maximum after 1 month from the beginning of infection. The antibody can persist for more than 1 year. Presence of specific IgM antibody in infected persons under 20 years of age is 80% but only 40% in subjects more than 20 years of age. During reinfection the antibody level rarely rises.

IgA: Specific IgA antibody usually increases later than IgM and often decreases earlier. Its significance becomes obvious when IgM antibody is absent in some patients or in case of reinfection.

IgG: Specific IgG antibody rises 2–3 weeks after symptoms appearance with maximum reached after longer period (about 6 months) and the antibody can persist for more than 1 year, in some cases even more than 4 years. In case of reinfection it is necessary to evaluate dynamics of antibodies by reinvestigation of paired samples collected in the course of 1 to 2 weeks.

It is advisable to examine each sample for all three antibody classes to evaluate the serological results, eventually to perform reinvestigation of paired samples.

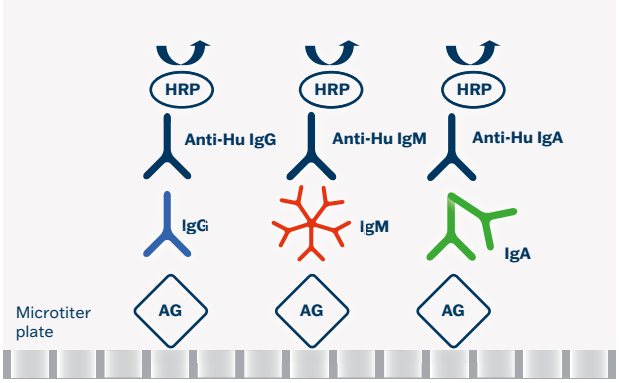
Antibody Responses



ELISA

Test Principle

The assays are based on a sandwich type of ELISA method.



Summary Protocol

Step		Test steps
	1.	Dilute samples – serum/plasma 1:101 (10 µl + 1 ml)
	2.	Pipette controls and diluted samples 100 µl – blank = empty well
	3.	Incubate 30 min. at 37 °C
	4.	Aspirate and wash the wells 5 times
	5.	Add 100 µl Conjugate – blank = empty well
	6.	Incubate 30 min. at 37 °C
	7.	Aspirate and wash the wells 5 times
	8.	Add 100 µl Substrate (TMB-Complete) – Including blank
	9.	Incubate 30 min. at 37 °C
	10.	Add 100 µl Stopping solution – Including blank
	11.	Read colour intensity at 450 nm

Antigens

EIA Mycoplasma

Purified and inactivated *M. pneumoniae* antigen enriched with highly specific immunodominant epitopes

EIA Mycoplasma REC

Mixture of highly specific recombinant antigens

Clinical Application

- Screening test for the detection of infection with Mycoplasma pneumoniae in humans
- Checking of therapy results using the semiquantitative or quantitative determination.

User Comfort

- Ready-to-use components
- Colour-coded components
- Interchangeable components
- Breakable colour-coded microplate strips
- CUT-OFF and Calibrators included
- Semiquantitative evaluation of results (Index of Positivity)
- Quantitative evaluation of results (U/ml)
- Easy assay procedure

Advantages

- Identical assay procedure
- High diagnostic specificity and sensitivity
- High reproducibility
- High dynamics of antibody response
- Expiration period of 15 months from date of production
- Short total assay time
- Ready for automation
- Customer support

Test Characteristics

ELISA	Diagnostic Sensitivity	Diagnostic Specificity
EIA Mycoplasma IgA	99.9%	84.6%
EIA Mycoplasma IgG	99.9%	85.7%
EIA Mycoplasma IgM	96.7%	98.8%
EIA Mycoplasma REC IgA	95.0%	95.4%
EIA Mycoplasma REC IgG	95.4%	95.5%
EIA Mycoplasma REC IgM	99.0%	97.7%

Types of Kits

SmartEIA kits are designed for automated processing using the Agility® analyser.

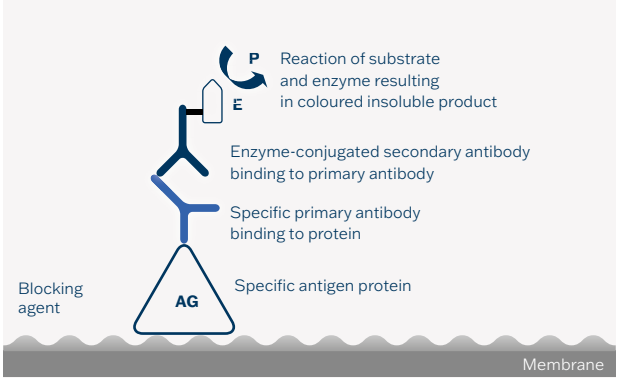
EIA

SmartEIA

IMMUNOBLOT

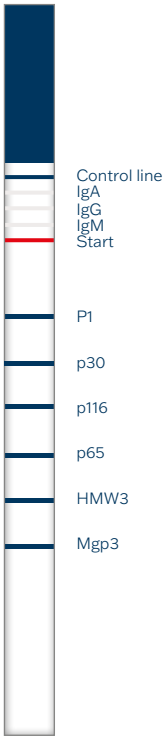
Test Principle

Recombinant antigens are transferred to a nitrocellulose membrane using a micro-dispensing method.



Antigens

BLOT-LINE Mycoplasma



P1 – Adhesin; the most important protein, a major virulence factor
p30 – Cytadhesin p30; the second most important protein, a major virulence factor
p116 – Adhesin, a major virulence factor
p65 – Surface protein; proline-rich P65 protein
HMW3 – Cytadherence high molecular weigh 3; adhesion-promoting protein
Mgp3 – Adhesion-promoting protein

Summary Protocol

Step		Test steps
	1.	Pipette Universal solution 2.5 ml
	2.	Strips soaking 10 min. at room temperature – Shaker
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (30 µl + 1.5 ml)
	5.	Pipette Controls and diluted samples 1.5 ml
	6.	Incubate 30 min. at room temperature – Shaker
	7.	Aspirate samples and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	8.	Pipette Conjugate 1.5 ml
	9.	Incubate 30 min. at room temperature – Shaker
	10.	Aspirate Conjugate and wash strips with 1.5 ml of Universal solution 3-times for 5 min. – Shaker
	11.	Pipette Substrate solution (BCIP/NBT) 1.5 ml
	12.	Incubate 15 min. at room temperature – Shaker
	13.	Aspirate Substrate solution and wash strips with 2 ml of distilled water 2-times for 5 min. – Shaker
	14.	Sticking and evaluation of strips

Clinical Application

- Detailed determination for the presence of anti-Mycoplasma specific antibodies
- Confirmation of ambiguous results
- Confirmation for ELISA tests

User Comfort

- Ready-to-use components
- Colour-coded strips
- Interchangeable components
- Positive and Negative controls
- Control line is present on the strip
- Possibility of software evaluation

IMMUNOBLOT

Advantages

- Identical assay procedure
- Easy interpretation and reproducibility of results
- Sophisticated evaluation software
- High diagnostic efficiency
- Ready for automation
- Customer support

Test Characteristics

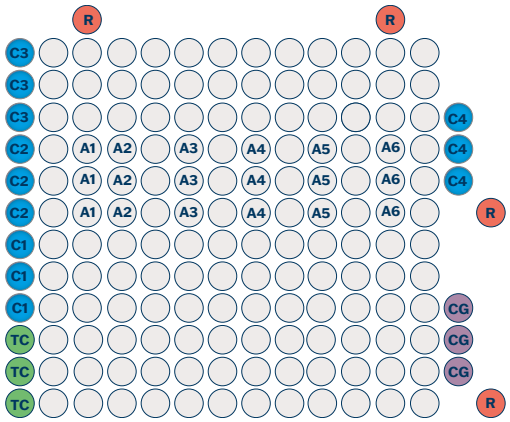
Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
BLOT-LINE Mycoplasma IgA	93.3%	93.7%
BLOT-LINE Mycoplasma IgG	92.4%	96.0%
BLOT-LINE Mycoplasma IgM	90.3%	95.1%

Interpretation Results

IgG	IgA	IgM	Interpretation
–	–	–	No serological evidence of Mycoplasma pneumoniae infection
–	+	+	Early phase of acute infection or reinfection
–	+	–	Early phase of acute infection or reinfection
+	+	+	Acute infection
+	–	+	Acute infection (late phase)
+	+	–	Reinfection or infection without IgM production
+	–	–	Past infection or reinfection
–	–	+	Early phase of acute infection

MICROBLOT-ARRAY

Distribution of Antigens and Control Spots



Description of antigens

A1 – P1
A2 – P30
A3 – P116
A4 – P65
A5 – HMW3
A6 – Mgp3

Description of control spots

R – Reference
TC – Test control
CA – Conjugate control IgA
CG – Conjugate control IgG
C1 – Calibration 1
C2 – Calibration 2
C3 – Calibration 3
C4 – Calibration 4

Protocol Summary

Step		Test steps
	1.	Pipette Universal solution 150 µl
	2.	Strips soaking 10 min. at room temperature
	3.	Aspirate
	4.	Dilute samples – serum/plasma 1:51 (10 µl + 500 µl)
	5.	Pipette Controls and diluted samples 100 µl
	6.	Incubate 30 min. at room temperature
	7.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	8.	Pipette Conjugate 100 µl
	9.	Incubate 30 min. at room temperature
	10.	Aspirate samples and wash strips with 150 µl of Universal solution 3-times for 5 min.
	11.	Pipette Substrate solution (BCIP/NBT) 100 µl
	12.	Incubate 15 min. at room temperature
	13.	Aspirate Substrate solution and wash strips with 200 µl of distilled water 2-times for 5 min.
	14.	Dry and evaluate strips

User Comfort

- Low sample consumption
- Antigens spotted in triplicate – minimizing statistical variation
- Possibility of automatic processing and results evaluation
- Parallel testing of multiple markers simultaneously
- High sensitivity and specificity

Microblot-Array

Test Characteristics

Pathogen	Diagnostic Sensitivity	Diagnostic Specificity
Microblot-Array Mycoplasma IgA	97.1%	99.3%
Microblot-Array Mycoplasma IgG	95.7%	99.0%

Cross-reactivity

of the kit Mycoplasma IgA on a panel of potentially cross-reactive samples

Category	n	Positive Result	Negative Result	Cross-reactivity
RF	22	1	21	4.6%
EBV	21	0	21	0.0%
ANA	22	1	21	4.6%
Chlamydia pneumoniae	23	1	22	4.4%
Bordetella pertussis	16	0	16	0.0%
CMV	15	0	15	0.0%
Legionella pneumophila	3	0	3	0.0%

of the kit Mycoplasma IgG on a panel of potentially cross-reactive samples

Category	n	Positive Result	Negative Result	Cross-reactivity
RF	22	1	21	0.0%
EBV	21	0	21	0.0%
ANA	22	1	21	0.0%
Chlamydia pneumoniae	23	1	22	0.0%
Bordetella pertussis	16	0	16	0.0%
CMV	15	0	15	0.0%
Legionella pneumophila	3	0	3	0.0%

Potential cross-reactivity with other related pathogens and factors is insignificant (less than 5%).

Ordering Information

ELISA

Cat. No.	Product	No. of Wells
MyA096	EIA Mycoplasma IgA	96
MyG096	EIA Mycoplasma IgG	96
MyM096	EIA Mycoplasma IgM	96
MyAR96	EIA Mycoplasma REC IgA	96
MyGR96	EIA Mycoplasma REC IgG	96
MyMR96	EIA Mycoplasma REC IgM	96
SK-MyA096	SmartEIA Mycoplasma IgA	96
SK-MyG096	SmartEIA Mycoplasma IgG	96
SK-MyM096	SmartEIA Mycoplasma IgM	96
SK-MyAR96	SmartEIA Mycoplasma REC IgA	96
SK-SK-MyGR96	SmartEIA Mycoplasma REC IgG	96
SK-MyMR96	SmartEIA Mycoplasma REC IgM	96

SmartEIA kits are designed for automated processing using the Agility® analyser

IMMUNOBLOT

Cat. No.	Product	No. of Tests
MyAL20	BLOT-LINE Mycoplasma IgA	20
MyGL20	BLOT-LINE Mycoplasma IgG	20
MyML20	BLOT-LINE Mycoplasma IgM	20
BD-MyAL24	BlueBLOT-LINE Mycoplasma IgA	24
BD-MyGL24	BlueBLOT-LINE Mycoplasma IgG	24
BD-MyML24	BlueBLOT-LINE Mycoplasma IgM	24
SwIm03	Immunoblot Software	1

BlueBLOT-LINE kits are designed for automatic processing using the BlueDiver analyser

Ordering Information

MICROBLOT-ARRAY

Cat. No.	Product	No. of Tests
MyAMA48	Microblot-Array Mycoplasma IgA	48
MyGMA48	Microblot-Array Mycoplasma IgG	48
MyMMA48	Microblot-Array Mycoplasma IgM	48

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Company is certified to the quality management system standards ISO 9001 and ISO 13485 for in vitro diagnostics.

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