DiaSorin Italia S.p.A.
Via Crescentino snc - 13040 Saluggia (VC) - Italy
www.diasorin.com
Tel. +39.0161.4871

CE 0459

Changes: §1, §2, §4, §5, §6, §7, §8, §9, §10, §12, §15.2, References;
Deletions: §14;

LIAISON® Mycoplasma pneumoniae IgM (REF 317030)

1. INTENDED PURPOSE

The LIAISON® Mycoplasma pneumoniae IgM assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro qualitative determination of specific IgM antibodies to *Mycoplasma pneumoniae* in human serum or plasma samples. The assay is intended as an aid in the diagnosis of current or past Mycoplasma pneumoniae infection in suspected patients. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Mycoplasma pneumoniae belongs to the *Mycoplasmataceae* family and Mollicutes class. All members of the Mollicutes class, including *M. pneumoniae*, are characterized by the lack of a cell wall, making these microorganisms resistant to β-lactam antibiotics that inhibit bacterial cell wall synthesis.[1]

Mycoplasma pneumoniae is the most common causative agent of primary atypical pneumonia, accounting for >50% of pediatric community-acquired pneumonia (CAP) and 32.5% of adult CAP.[2] Most *M. pneumoniae* infections lead to a self-limiting clinical disease, but 1 to 5% of cases may require hospitalization.[4] The most common symptoms are: fever, headache, cough, myalgias, runny nose and gastrointestinal symptoms.[3,4] *M. pneumoniae* infection can also result in extrapulmonary manifestations, even in the absence of respiratory symptoms. Although rare, complications can affect the central nervous system and may also result in dermatological, hematological and cardiac manifestations.

Mycoplasmas are not considered obligatory intracellular residents but they rely on a host cell to provide the necessary nutrients.[5,6] To bind the host cell, *M. pneumoniae* has an apical organelle with several binding proteins: P1 (170 kDa), P30 (30 kDa), P116 (116 kDa), HMW1-3 and proteins A, B and C. Among these, P1 and P30, in addition to being linked to the receptor adhesion process, are also responsible for the host immune response.[6]

M. pneumoniae infections occur endemically and epidemically worldwide, regardless of geographical location, and can occur in people of all ages.[5] Infection mortality is low, but a high mortality rate (30%) has been reported in the elderly.[3] People of all ages can get *M. pneumoniae* infection, but it is more common among young adults and school-age children.[12] *M. pneumoniae* infections usually spread among families living in the same house, as well as people who work or live in crowded places such as schools, nursing homes and hospitals.[5,9]

Mycoplasma pneumoniae infection is known as “walking pneumonia” due to the supposed benign nature of the disease and is usually self-limiting. However, in some cases, it may cause a more serious clinical picture.[3] Symptoms such as malaise, fever, myalgia and persistent non-productive cough may be present. Pneumonia is the most severe clinical manifestation, occurring mainly in children older than five years.[8] Many of these symptoms are common to other respiratory diseases.[7,10] To improve the identification of patients in need of treatment and to avoid unnecessary antibiotic therapy, early diagnosis of *M. pneumoniae* infection is essential.[7,11]

The diagnosis of *M. pneumoniae* infection depends mainly on microbiological and serological assays.[15] *M. pneumoniae* is difficult to cultivate, therefore, for microbiologic diagnosis, the polymerase chain reaction (PCR) assay is most commonly used. Even so, a negative culture or PCR does not exclude the possibility of infection, as *M. pneumoniae* goes through varying periods after acute infection and may or may not be detectable.[13,14]

Antibody production against *M. pneumoniae* antigens can be detected one week after infection, peaking within 3 to 6 weeks, with a gradual decline that can last months to years.[1,5] The first class of antibodies that appear are from immunoglobulin M (IgM). IgM increases 7-10 days after symptom onset, peaking after 4-6 weeks and gradually decreasing, becoming undetectable after 12-26 weeks in 76.5% of patients, although it may persist for up to twelve months. Tests in the first week after infection may result in false negative results.[1,12] Immunoglobulins G (IgG) appear 2 to 3 weeks after the onset of IgM, peaking at approximately the fifth week after disease onset, and are considered a reliable marker of *M. pneumoniae* infection. In order to increase the sensitivity of the serological diagnosis, it is recommended to perform two serum tests. A fourfold increase in *M. pneumoniae*-specific IgG titers obtained in the 2-4 week interval between the acute and convalescent phases or an increase in specific IgM antibodies indicates *M. pneumoniae* infection.[3,5,12]

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of specific IgM to *Mycoplasma pneumoniae* is an indirect *sandwich* chemiluminescence immunoassay (CLIA). Mycoplasmal lysate enriched with recombinant antigen, that provides high specificity and sensitivity, is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody directed against human IgM is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, calibrators, samples or controls are diluted with buffer A, which contains goat IgG to human IgG as an absorbent reagent to curb interference from human IgG specific to *Mycoplasma pneumoniae* or from rheumatoid factor. *Mycoplasma pneumoniae* antibodies – if present in calibrators, samples or controls – bind to the solid phase. During the second incubation, the antibody conjugate reacts with any human anti-*Mycoplasma pneumoniae* IgM already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.
Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and indicates the presence or absence of IgM to *Mycoplasma pneumoniae* in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (1.6 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with mycoplasmal lysate (approx. 150 µg/mL) enriched with recombinant peptide P1 (obtained in *E. coli*) (approx. 150 µg/mL), BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (0.6 mL)	CAL 1	BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (approx. 1.5 Index) are referenced to an in-house antibody preparation.
Calibrator 2 (0.6 mL)	CAL 2	Recombinant IgM antibodies reactive against *Mycoplasma Pneumoniae* (approx. 11.5 Index), BSA, human serum/plasma non-reactive for *Mycoplasma pneumoniae* IgM (approx. 10%), phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300 and an inert blue dye. The calibrator concentrations are referenced to an in-house antibody preparation.
Buffer A (15.8 mL)	BUF A	Goat IgG to human IgG (absorbent reagent) (≥ 5%), goat serum, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert blue dye.
Conjugate (13.5 mL)	CONJ	Mouse monoclonal antibodies to human IgM conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives, an inert yellow dye.
Number of tests		50

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the reagent integral.

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® Analyzer
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015) or LIAISON® Disposable Tips (REF X0055). LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300) LIAISON® Wash/System Liquid (REF 319100). LIAISON® XL Waste Bags (REF X0025). LIAISON® XL Cleaning Tool (REF 310995) or LIAISON® EASY Cleaning Tool (REF 310996)	LIAISON® Module (REF 319130). - - LIAISON® Starter Kit (REF 319102) or LIAISON® XL Starter Kit (REF 319200) or LIAISON® EASY Starter Kit (REF 319300) LIAISON® Light Check 12 (REF 319150). LIAISON® Wash/System Liquid (REF 319100). LIAISON® Waste Bags (REF 450003). LIAISON® Cleaning Kit (REF 310990). -

LIAISON® XS Analyzer
LIAISON® Cuvettes on Tray (REF X0053). LIAISON® Disposable Tips (REF X0055). LIAISON® EASY Starter Kit (REF 319300). LIAISON® EASY Wash Buffer (REF 319301). LIAISON® EASY System Liquid (REF 319302). LIAISON® EASY Waste (REF X0054). LIAISON® EASY Cleaning Tool (REF 310996).

Additionally required materials

LIAISON® Mycoplasma pneumoniae IgM controls (negative and positive) (REF 317031).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use. For Laboratory Professional Use Only.

Visually inspect the integral vials for leaking at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

All serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

The analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.

Do not use kits or components beyond the expiration date given on the label.

Pursuant to EC Regulation 1272/2008 (CLP) hazardous reagents are classified and labeled as follows:

REAGENTS:	CAL 1, CAL 2, BUF A, CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request.
For additional information see Safety Data Sheets available on www.diasorin.com.

7. REAGENT PREPARATION

REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:
Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Carefully wipe the surface of each septum to remove residual liquid.
Repeat as necessary until the magnetic particles are completely resuspended.
Incomplete magnetic particle resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:
Visually inspect the reagents, calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the integral. If foam is present after resuspension of the magnetic particles, place the integral on the instrument and allow the foam to dissipate. The integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.

Loading of integral into the reagent area

LIAISON® Analyzer

– Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
– Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS analyzers

– LIAISON® XL and LIAISON® XS analyzers are equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
– Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.

Follow the analyzer operator's manual to load the specimens and start the run

CONTROLS

Refer to the LIAISON® Mycoplasma pneumoniae IgM Control Set instructions for use section for proper preparation and handling instructions.

8. REAGENT INTEGRAL STORAGE AND STABILITY

– **Sealed**: Stable at 2-8°C until the expiry date.
– **Opened on board or at 2-8°C**: eight (8) weeks.
– Use always the same analyzer for a reagent integral already opened.
– Use storage rack provided with the analyzer for upright storage of reagent integral.
– Do not freeze.
– Keep upright for storage to facilitate later proper resuspension of magnetic particles.
– Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

The correct type of specimen must be used with the assay. The following have been tested and may be used:
– Serum;
– Plasma collected with the following anticoagulant:
.Sodium heparin;
.potassium EDTA;
.sodium citrate.

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.
Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.
Package and label specimens in compliance with applicable regulations covering the transport of clinical specimens and infectious substances.
Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below.
Uncontrolled transport conditions (in terms of temperature and time) may cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore, not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010).
A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells or gel separator. The following storage conditions showed no significant differences:
- room temperature storage should be avoided;
- 2°-8°C for eight (8) days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
- Up to five (5) freeze-thaw cycles, however multiple freeze thaw cycles should be avoided.

If samples are stored frozen, mix thawed samples well before testing.
Further centrifugation of specimens removed from red cells, clot or gel separator (suggested between 3,000 and 10,000 g for 10 minutes) is recommended to guarantee the consistency of results whenever one of the following conditions is identified:
- Samples previously centrifuged and stored at 2°-8°C;
- Samples with particulate matter, fibrin, turbidity, lipaemia or erythrocyte debris;
- Samples frozen and thawed;
- Samples requiring repeat testing.

Specimens with a lipid layer on the top should be transferred into a secondary tube, taking care to transfer only the clarified material. Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Heat inactivation of the specimens may affect the test results. Check for and remove air bubbles before assaying.
The minimum volume required for a single determination is 170 µL of specimen (20 µL specimen + 150 µL dead volume).

10. CALIBRATION

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows four (4) calibrations to be performed.
Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
– A new lot of Starter Kit is used.
– The previous calibration was performed more than eight (8) weeks before.
– Each time a new lot of integral is used.
– **LIAISON® and LIAISON® XL analyzers:** the Analyzer has been serviced.
– **LIAISON® XS Analyzer**: After a technical intervention, only if required by the service procedure, as communicated by local DiaSorin technical support or representative.
– Control values lie outside the expected ranges.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
LIAISON® XL Analyzer: Calibrator values are stored in the Radio Frequency IDentification transponder (RFID Tag).
LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency IDentification transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL and LIAISON® XS analyzers. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

The analyzer operations are as follows:

1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense buffer A and coated magnetic particles.
3. Incubate.
4. Wash with Wash/System liquid.
5. Dispense conjugate into the reaction module.
6. Incubate.
7. Wash with Wash/System liquid.
8. Add the Starter Kit and measure the light emitted.

Warning - *Maintenance with the LIAISON® XL Cleaning Tool (REF 310995) or LIAISON® EASY Cleaning Tool (REF 310996) must be performed (refer to LIAISON® XL Cleaning Tool instruction for use for details).*

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Control Mycoplasma pneumoniae IgM (REF 317031)
(a) at least once per day of use,
(b) whenever a new reagent integral is used,
(c) whenever the kit is calibrated,
(d) whenever a new lot of Starter Reagents is used,
(e) to assess adequacy of performance of the open integral beyond eight (8) weeks, or in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates *Mycoplasma pneumoniae* IgM levels expressed as index value and grades the results. For details, refer to the analyzer operator's manual.

Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS but patient results are equivalent.

Assay range. 0.1 to 27 index value *Mycoplasma pneumoniae* IgM.

13.1. Interpretation of results obtained in single specimens

For reliable interpretation of results both IgG and IgM antibodies must be tested.

The cut-off value discriminating between the presence and the absence of *Mycoplasma pneumoniae* IgM has an index value of 10. Sample results should be interpreted as follows:
Samples with *Mycoplasma pneumoniae* IgM levels below an index value of 10 should be graded *negative*.
Samples with *Mycoplasma pneumoniae* IgM levels equal to or above an index value of 10 should be graded *positive*.

IgM index value	Classification	Interpretation of results
< 10	negative	No evidence of infection. A negative result for IgM antibodies to *Mycoplasma pneumoniae* generally indicates that the patient has not been infected, but does not exclude the possibility of infection. Adults who have been infected repeatedly over a period of years may not respond to mycoplasmal antigens with a strong IgM response.
≥ 10	positive	Probable acute infection.

13.2. Interpretation of IgG and IgM combined results obtained in single specimens

IgG result	IgM result	Interpretation of results
negative	negative	No evidence of infection. A negative result for IgM antibodies to *Mycoplasma pneumoniae* generally indicates that the patient has not been infected, but does not exclude the possibility of infection. Adults who have been infected repeatedly over a period of years may not respond to mycoplasmal antigens with a strong IgM response.
negative or positive	positive	Test a second sample, collected two to four weeks later in parallel with the first sample.
positive	negative	Test a second sample, collected two to four weeks later in parallel with the first sample.

14. LIMITATIONS OF THE PROCEDURE

– Assay performance characteristics have not been established when any LIAISON® Mycoplasma pneumoniae test is used in conjunction with other manufacturers' assays for detection of specific *Mycoplasma pneumoniae* serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.
– A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.
– Bacterial contamination or heat inactivation of the specimens may affect the test results.
– This test is suitable only for investigating single samples, not sample pools.
– Test results are reported qualitatively as positive or negative for the presence of *Mycoplasma pneumoniae* IgM. However, diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgement.
– Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may interfere in a monoclonal antibody-based immunoassay and their results should be evaluated with care.
– Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted.

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, potassium EDTA, sodium heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), albuminaemia (up to 5.0 g/dL albumin). Results are not altered by up to five freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® Mycoplasma pneumoniae IgM assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to those of *Mycoplasma pneumoniae* infection (adenovirus, influenza A and B viruses, parainfluenza viruses, respiratory syncytial virus, *Bordetella pertussis, Chlamydia pneumoniae, Legionella pneumophila*), from antibodies to other organisms that may cause infectious diseases (EBV, hCMV, parvovirus B19, *Toxoplasma gondii, Treponema pallidum*) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor, HAMA or human anti-mouse antibodies). Samples for these studies were pre-screened with another commercially available *Mycoplasma pneumoniae* IgM assay. If found negative for *Mycoplasma pneumoniae* IgM antibodies, those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays. The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Clinical condition	N.	LIAISON®		Kit A		Kit B	
		+	%	+	%	+	%
hCMV IgM antibodies	10	2	20	0	0	2	20
EBV (VCA) IgM antibodies	34	9	26	2	6	6	18
Parvovirus B19 IgM antibodies	18	2	11	1	6	5	28
Adenovirus antibodies	5	0	0	0	0	0	0
Influenza A and B viruses antibodies	10	2	20	0	0	2	20
Parainfluenza viruses antibodies	4	0	0	0	0	0	0
Respiratory syncytial virus (RSV) IgM antibodies	5	0	0	0	0	0	0
Bordetella pertussis IgM antibodies	5	0	0	0	0	0	0
Chlamydia pneumoniae IgM antibodies	5	0	0	2	40	2	40
Legionella pneumophila IgM antibodies	5	0	0	0	0	0	0
Toxoplasma gondii antibodies	9	0	0	1	11	0	0
Treponema pallidum antibodies	5	0	0	0	0	0	0
Rheumatoid factor (anti-Fc immunoglobulin)	5	1	20	0	0	1	20
Anti-nuclear autoantibodies (ANA)	5	0	0	0	0	0	0
Human anti-mouse antibodies (HAMA)	5	0	0	1	20	0	0
Total	130	16	12%	7	5%	18	14%

As reported in the literature (1) commercial assays for detection of *Mycoplasma pneumoniae* IgM may show false-positive results due to the presence of IgM to other etiological agents and not clearly related to the presence or absence of heterophile antibodies. Such reactivity may be relevant in clinical practice.

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Repeatability. Twenty replicates were performed in the same run to evaluate in-house repeatability.

Repeatability	A	B	E	C	D
Number of determinations	20	20	20	20	20
Mean (index value)	4.1	4.3	16.1	18.3	26.3
Standard deviation (index value)	0.1	0.2	0.7	0.6	0.8
Coefficient of variation (%)	3.1	5.7	4.5	3.3	3.2
Min. value (index value)	3.9	3.9	14	17	24
Max. value (index value)	4.3	4.7	18	20	27

Reproducibility. Twenty replicates were performed in different days (maximum of two runs per day) with three different lots of integral to evaluate reproducibility. The tests were performed in two sites, in house (site 1) and in an independent laboratory (site 2) using two different instruments.

Reproducibility - Site 1	A	B	E	C	D
LOT No. 01					
Number of determinations	20	20	20	20	20
Mean (index value)	4.4	4.4	14.1	17.1	23.6
Standard deviation (index value)	0.4	0.3	1.6	0.9	1.9
Coefficient of variation (%)	8.5	6.9	11.3	5.3	8.0
Min. value (index value)	3.1	3.9	10	15	20
Max. value (index value)	4.9	5.1	17	19	26
LOT No. 02					
Number of determinations	20	20	20	20	20
Mean (index value)	4.7	3.9	14.7	17.4	23.3
Standard deviation (index value)	0.4	0.2	1.6	1.3	1.4
Coefficient of variation (%)	8.7	5.6	11.1	7.3	6.1
Min. value (index value)	4.1	3.6	10.0	14	21
Max. value (index value)	5.4	4.5	18	20	25
LOT No. 03					
Number of determinations	20	20	20	20	20
Mean (index value)	4.5	4.0	15.8	16.9	22.8
Standard deviation (index value)	0.6	0.3	1.9	1.8	1.2
Coefficient of variation (%)	13.3	7.6	11.9	10.8	5.5
Min. value (index value)	3.1	3.5	11	14	20
Max. value (index value)	6.2	4.7	19	23	25
Inter-lot coefficient of variation (%)	3.5	7.1	6.1	1.7	1.7

Reproducibility - Site 2	A	B	E	C	D
LOT No. 01					
Number of determinations	20	20	20	20	20
Mean (index value)	4.1	4.1	12.9	17.5	23.1
Standard deviation (index value)	0.4	0.3	1.1	0.9	1.9
Coefficient of variation (%)	11.0	8.1	8.2	5.4	8.2
Min. value (index value)	3.2	3.6	11	15	20
Max. value (index value)	4.8	4.8	15	19	27
LOT No. 02					
Number of determinations	20	20	20	20	20
Mean (index value)	4.0	3.6	12.7	17.1	22.8
Standard deviation (index value)	0.4	0.3	1.1	1.0	1.3
Coefficient of variation (%)	9.9	7.8	8.4	5.9	5.7
Min. value (index value)	3.3	3.1	11	15	21
Max. value (index value)	4.5	4.1	14	19	26
LOT No. 03					
Number of determinations	20	20	20	20	20
Mean (index value)	3.7	3.6	12.0	14.9	20.0
Standard deviation (index value)	0.7	0.6	2.0	2.5	3.0
Coefficient of variation (%)	19.0	17.5	16.3	17.1	14.9
Min. value (index value)	2.2	2.4	8.2	10	14
Max. value (index value)	4.3	4.5	14	18	25
Inter-lot coefficient of variation (%)	6.1	6.0	3.7	8.5	7.7

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Repeatability. Twenty replicates were performed in the same run to evaluate in-house repeatability.

Repeatability	1	2	3	Negative control	Positive control
Number of determinations	20	20	20	20	20
Mean (index value)	3.9	12.6	21.3	1.1	15.3
Standard deviation	0.27	0.90	0.84	0.074	0.93
Coefficient of variation (%)	7.1	7.1	3.9	6.9	6.1
Min. value (index value)	3.4	11	19	0.9	13
Max. value (index value)	4.4	14	23	1.2	17

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) on one integral lot to evaluate in-house reproducibility.

Reproducibility	4	5	6	Negative control	Positive control
Number of determinations	20	20	20	20	20
Mean (index value)	6.5	13.8	15.1	1.0	14.7
Standard deviation	0.45	1.2	1.3	0.15	1.5
Coefficient of variation (%)	7.0	8.4	8.6	15.2	10.3
Min. value (index value)	5.6	11	12	0.7	12
Max. value (index value)	7.6	15	17	1.2	18

15.4. Precision with LIAISON® XS Analyzer

A five day precision study was conducted on three LIAISON® XS Analyzers to verify the precision with the LIAISON® Mycoplasma pneumoniae IgM Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol. A coded panel comprised of 7 frozen samples was used for the study.
The LIAISON® Control Mycoplasma pneumoniae IgM set was also included in the five day study.
The coded panel was tested on three LIAISON® XS Analyzers, in six replicates in a single run per day, for 5 operative days.
The dose mean, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same test to evaluate repeatability. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot..

Repeatability	7	8	9	10	11	12	13	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	1.7	7.2	7.5	14.2	18.2	11.8	15.0	1.6	14.7
Standard deviation	0.076	0.203	0.187	0.558	0.529	0.450	0.651	0.094	0.386
Min. value (index value)	1.3	6.2	6.3	12.0	16.0	10.0	13.0	1.3	13.0
Max. value (index value)	2.0	8.5	8.4	18.0	22.0	14.0	18.0	2.4	17.0
Coefficient of variation (%)	4.4	2.8	2.5	3.9	2.9	3.8	4.3	5.6	2.6

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 7 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

Reproducibility	7	8	9	10	11	12	13	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	1.7	7.2	7.5	14.2	18.2	11.8	15.0	1.6	14.7
Standard deviation	0.137	0.478	0.457	0.895	1.198	0.737	0.945	0.149	0.594
Min. value (index value)	1.3	6.2	6.3	12.0	16.0	10.0	13.0	1.3	13.0
Max. value (index value)	2.0	8.5	8.4	18.0	22.0	14.0	18.0	2.4	17.0
Coefficient of variation (%)	8.0	6.6	6.1	6.3	6.5	6.2	6.3	9.0	4.0

15.5. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve).
Analysis of saturation effect was evaluated by testing three high-titred samples positive for *Mycoplasma pneumoniae* IgM. All samples resulted in high concentration values as expected, indicating no sample misclassification.

15.6. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 445 specimens belonging to a population with signs and symptoms of atypical pneumonia, collected in different laboratories. The specimens were also tested by a reference ELISA method and consensus with additional serological data was applied to define the expected results for discrepant specimens (36/445). Twelve specimens were unresolved by the reference method and therefore were not included in the data analysis. The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

Agreement with reference method

Agreement for positive results: 97.0% (96/99) (95% confidence interval: 91.4-99.4%).
Agreement for negative results: 94.3% (313/332) (95% confidence interval: 91.2-96.5%).
Overall agreement: 91.9% (409/445) (95% confidence interval: 89.0-94.3%).

Results after consensus

Seven positive and 315 negative results were observed in the expected negative population studied - diagnostic specificity: 97.8% (315/322) (95% confidence interval: 95.6-99.1%).

One negative and 110 positive results were observed in the expected positive population studied - diagnostic sensitivity: 99.1% (110/111) (95% confidence interval: 95.0-100%).

For EU only: please be aware that any serious incident that has occurred in relation to this IVD medical device should be reported to DiaSorin Italia S.p.A. and to the Competent Authority of the EU Member State in which the user and/or the patient is established.

REFERENCES

1 Sobieszczanska, B. M. et al. Relevance of serology for Mycoplasma pneumoniae infection among children with persistent cough. Adv Clin Exp Med 23, 185-190, (2014).

2 Wijesooriya, L. I., Kok, T., Perera, J., Tilakarathne, Y. & Sunil-Chandra, N. P. Mycoplasma pneumoniae DNA detection and specific antibody class response in patients from two tertiary care hospitals in tropical Sri Lanka. J Med Microbiol 67, 1232-1242, (2018).

3 Bajantri, B., et al. Mycoplasma pneumoniae: A Potentially Severe Infection. J Clin Med Res 10, 535-544, (2018).

4 ECDC. Surveillance status and recent data for Mycoplasma pneumoniae infections in the European Union and European Economic Area, January 2012. Lenglet A, Herrador Z, Magiorakos AP, Leitmeyer K, Coulombier D 17, (2012).

5 Atkinson, P. T., et al. Epidemiology, clinical manifestations, pathogenesis and laboratory detection of Mycoplasma pneumoniae infections. FEMS Microbiol Rev 32, 956-973, (2008).

6 Tabassum, I., Chaudhry, R., Chourasia, B. K. & Malhotra, P. Identification of an N-terminal 27 kDa fragment of Mycoplasma pneumoniae P116 protein as specific immunogen in M. pneumoniae infections. BMC Infect Dis 10, 350, (2010).

7 Dumke, R. et al. Optimized serodiagnosis of Mycoplasma pneumoniae infections. Diagn Microbiol Infect Dis 73, 200-203, (2012).

8 Atkinson, P. T., et al. Mycoplasma pneumoniae Infections in Childhood. The Pediatric Infectious Disease Journal 33, (2014).

9 CDC. Mycoplasma pneumoniae Infections, <https://www.cdc.gov/pneumonia/atypical/mycoplasma/index.html> (2018).

10 Du, D. et al. Serological Analysis and Drug Resistance of Chlamydia pneumoniae and Mycoplasma pneumoniae in 4500 Healthy Subjects in Shenzhen, China. Biomed Res Int 2017, 3120138, (2017).

11 Kumar, S., Garg, I. B., Sethi, G. R., Kumar, S. & Saigal, S. R. Detection of immunoglobulin M and immunoglobulin G antibodies to Mycoplasma pneumoniae in children with community-acquired lower respiratory tract infections. Indian J Pathol Microbiol 61, 214-218, (2018).

12 Zhang, Y. et al. Simultaneous detection of Mycoplasma pneumoniae IgG and IgM using dual-label time resolved fluoroimmunoassay. Anal Biochem 548, 1-6, (2018).

13 Dumke, R., Von Baum, H., Luck, P. C. & Jacobs, E. Subtypes and variants of Mycoplasma pneumoniae: local and temporal changes in Germany 2003-2006 and absence of a correlation between the genotype in the respiratory tract and the occurrence of genotype-specific antibodies in the sera of infected patients. Epidemiol Infect 138, 1829-1837, (2010).

14 She, R. C. et al. Limited utility of culture for Mycoplasma pneumoniae and Chlamydophila pneumoniae for diagnosis of respiratory tract infections. J Clin Microbiol 48, 3380-3382, (2010).

15 Gao, C. H., Ji, B. J., Han, C. & Wang, M. S. Comparison of enzyme-linked immunosorbent assay with indirect immunofluorescence assay for the diagnosis of Mycoplasma pneumoniae infection. J Clin Lab Anal 33, e22677, doi:10.1002/jcla.22677 (2019).

16. Bowen et al., Clinical Biochemistry, 43, 4-25, 2010

17. CLSI document EP15-A3 and CLSI EP17-A2,

200/007-941, 13 - 2025-04