

Changes: §1, §2, §4, §5, §6, §7, §8, §9, §10, §12, §14, §15.2, §15.3, §15.4, §15.5, References;
Deletions: §14;

LIAISON® Mumps IgM (REF 318830)

1. INTENDED PURPOSE

The LIAISON® Mumps IgM assay uses **chemiluminescent** immunoassay (CLIA) technology for the **in vitro** qualitative determination of specific IgM antibodies to Mumps virus in human serum or plasma samples. **This assay is intended as an aid in the determination of immune status to Mumps and as an aid in the diagnosis of infection of Mumps virus.**

The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Mumps virus (MuV) is a member of the *Rubulavirus* genus which belongs to the family of Paramyxoviridae, with other members that include measles virus, respiratory syncytial virus and parainfluenza virus^{1,2}. Humans are the only natural host of MuV¹. Transmission of mumps is possible from an infected person between 3-7 days before to 4-5 days after the onset of parotitis, but asymptomatic individuals can also shed and spread the disease^{2,7,17}. Viral transfer takes place by inhalation or oral contact with infected respiratory droplets or secretions, saliva or fomites^{1,14}. Mumps is one of the vaccine-preventable diseases, it is a part of measles, mumps, rubella [MMR] vaccination. Before vaccination, 95% of adults had serological evidence of past exposure, mostly from acquisition during childhood. After the implementation of mass vaccination, incidence dropped to less than 1/1 000 000 in the general population⁶. However, large sporadic outbreaks have continued to emerge, affecting even those who had been vaccinated. In contrast to the rubella and measles vaccines, the mumps vaccine is less effective, with roughly 78% seroconversion after one dose and 88% after two doses^{4,6}.

The diagnosis of mumps is largely based on clinical observations but definite diagnosis requires laboratory data because a large number of other viral and bacterial infections can cause the symptoms of parotitis and orchitis¹³. Anti-MuV IgM antibodies in serum form a commonly applied marker to diagnose mumps infection and are particularly useful during the initial period of illness^{2,5,8,14-16}. Some caution is advised because IgM titers may not be detectable in infected individuals with a history of one- or multiple-dose vaccination^{2,3,5}. The sensitivity of IgM in the diagnosis of mumps is 24-51% for samples collected in the first ten days after onset of symptoms and rises to 80-100% for samples collected after ten days³. IgM titers remain elevated for 4-8 weeks after onset of symptoms³. A rise in mumps-specific IgG titers is also associated with mumps infection. To detect such an elevation in IgG levels, acute and convalescent sera are needed and a relative increase, usually set to be at minimum fourfold, is considered to indicate an active mumps infection^{2,5}. Both anti-MuV IgG and IgM antibodies are routinely used in clinical practice to confirm the diagnosis of mumps in patients presenting with signs and symptoms of the disease, including parotitis⁹, orchitis^{10,11} and acute encephalopathy¹². Exposure to the mumps virus induces an antibody response that persist long after the infection subsides, usually lifelong. Although no perfect correlate exists for mumps immunity, the presence of (IgG) antibodies is generally considered to adequately reflect immunity of an individual to mumps. In individuals at risk for mumps infection due to increased vulnerability or increased exposure risk and without knowledge of immunization status, a serological analysis is recommended. Guidelines consider the presence of anti-mumps virus IgM antibodies and a fourfold or greater increase in anti-mumps virus IgG antibodies to be diagnostic for mumps infection.

3. PRINCIPLE OF THE PROCEDURE

The method for qualitative determination of specific IgM to mumps virus an indirect *sandwich* chemiluminescence immunoassay (CLIA). Recombinant mumps virus antigen 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 mumps virus or from rheumatoid factor. Mumps virus 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-mumps virus 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 mumps virus in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (1.6 mL)	[SORB]	Magnetic particles ($\geq 0.25\%$ solid) coated with recombinant mumps virus nucleoprotein (approx. 12.5 µg/mL) (obtained in <i>Pichia pastoris</i>), BSA, PBS buffer < 0.1% sodium azide.
Calibrator 1 (0.55 mL)	[CAL1]	Human serum/defibrinated plasma non-reactive for mumps virus IgM (approx. 0.3 Index), BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (index value) are referenced to an in-house antibody preparation.
Calibrator 2 (0.55 mL)	[CAL2]	HuCAL® IgM Antibodies (under license from MorphoSys AG. HuCAL® is a registered trademark of MorphoSys AG) reactive for mumps virus (approx. 2.0 Index), Human serum/defibrinated plasma non-reactive for mumps virus IgM, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (index value) are referenced to an in-house antibody preparation.
Buffer A (21 mL)	[BUFA]	Goat IgG to human IgG (absorbent reagent) ($\geq 5\%$), 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 (minimum 10 ng/mL) conjugated to an isoluminol derivative, 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® Module ([REF] 319130).
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® 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® Wash/System Liquid ([REF] 319100).
LIAISON® XL Waste Bags ([REF] X0025).	LIAISON® Waste Bags ([REF] 450003).
LIAISON® XL Cleaning Tool ([REF] 310995) or LIAISON® EASY Cleaning Tool ([REF] 310996).	LIAISON® Cleaning Kit ([REF] 310990).
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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® Mumps IgM controls (negative and positive) (**[REF]** 318831).

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:	CAL1, CAL2, BUFA, 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. PREPARATION OF REAGENT INTEGRAL

Please note the following important reagent handling precautions:

WARNING: Perform gentle mixing of the reagent integral before performing any calibration. In detail, before using the reagent integral for a calibration, please gently and carefully mix making an oscillating movement of the integral with the wrist so that it makes an angle of 180° overall (for approximately 10 seconds), avoiding foaming. In case of reagent integrals already in use (with seals removed), please slowly mix the integral carefully so that no liquid escapes from the vials' protective septums. Please, contact your DiaSorin Representative for further information.

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.

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 Analyzer and LIAISON® XS Analyzer 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.

8. REAGENT INTEGRAL STORAGE AND STABILITY

- **Sealed:** Stable at 2-8°C until the expiry date.
- **Opened on board or at 2-8°C:** Minimum stability 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:
 - .heparin;
 - .potassium EDTA.

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 7 days, otherwise they should be aliquoted and stored deep-frozen (-20°C or below);
- Up to 6 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 calibrations to be performed.

WARNING: Perform gentle mixing of the reagent integral before performing any calibration. In detail, before using the reagent integral for a calibration, please gently and carefully mix making an oscillating movement of the integral with the wrist so that it makes an angle of 180° overall (for approximately 10 seconds), avoiding foaming. In case of reagent integrals already in use (with seals removed), please slowly mix the integral carefully so that no liquid escapes from the vials' protective septums. Please, contact your DiaSorin Representative for further information.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

- A new lot of reagent integral or of Starter Kit is used.
- The previous calibration was performed more than four (4) weeks before.
- Control values lie outside the expected ranges.
- **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 DiaSorin Technical support or representative.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.

LIAISON® XL and LIAISON® XS analyzers: 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 specimen diluent.
3. Dispense coated magnetic particles.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. 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 pertinent 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® Mumps IgM controls ([REF 318831](#))

- (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 mumps virus IgM concentrations 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® and LIAISON® XL, but patient results are equivalent.

On LIAISON® Analyzer

Assay range. 0.5 to 4 index value mumps virus IgM.

The cut-off value discriminating between the presence and the absence of mumps virus IgM is 1.0 index value. Sample results should be interpreted as follows:

Samples with mumps virus IgM concentrations below 0.9 index value should be graded *negative*.

Samples with mumps virus IgM concentrations ranging between 0.9 and 1.1 index value should be graded *equivocal*. *Equivocal samples must be retested in order to confirm the initial result. Samples which are positive at the second test should be considered positive. Samples which are negative at the second test should be considered negative. A second sample should be collected and tested no less than one to two weeks later when the result is repeatedly equivocal.*

Samples with mumps virus IgM concentrations equal to or above 1.1 index value should be graded *positive*.

On LIAISON® XL Analyzer and LIAISON® XS Analyzer

The cut-off value discriminating between the presence and the absence of mumps virus IgM is 1.0 index value. Sample results should be interpreted as follows:

Samples with mumps virus IgM concentrations below 0.9 index value should be graded *negative*.

Samples with mumps virus IgM concentrations ranging between 0.9 and 1.1 index value should be graded *equivocal*. *Equivocal samples must be retested in order to confirm the initial result. Samples which are positive at the second test should be considered positive. Samples which are negative at the second test should be considered negative. A second sample should be collected and tested no less than one to two weeks later when the result is repeatedly equivocal.*

Samples with mumps virus IgM concentrations equal to or above 1.1 index value should be graded *positive*.

A positive result is generally indicative of acute infection. A negative result, however, does not always rule out acute mumps virus, because the infection is in its very early stage and the patient may be still unable to synthesize mumps virus specific IgM. If clinical exposure to mumps virus is suspected despite a negative finding, a second sample should be collected and tested no less than one or two weeks later. An equivocal result is indicative either of recent infection or of past infection with long-lasting mumps virus IgM. Serological data from detection of additional mumps virus markers may provide useful information for clinical interpretation of results.

14. LIMITATIONS OF THE PROCEDURE

- 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.
- Test results are reported qualitatively as positive or negative for the presence of mumps virus 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.
- The presence of infectious mononucleosis must be excluded in patients with isolated positive result for mumps virus IgM. Polyclonal stimulation of B lymphocytes during infectious mononucleosis, in fact, may result in non-specific induction of synthesis of mumps antibodies, especially of the IgM class.
- **Results obtained with LIAISON® Mumps IgM assay may not be used interchangeably with values obtained with different manufacturers' assay methods.** Assay performance characteristics have not been established when any LIAISON® mumps test is used in conjunction with other manufacturers' assays for detection of specific mumps serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.
- 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 (potassium EDTA, heparin, sodium citrate), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin) by up to six freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® Mumps IgM assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to those of mumps (hCMV, EBV, rubella virus, parvovirus B19, measles virus, *Toxoplasma gondii*, parainfluenza viruses), from antibodies to other organisms that may cause infectious diseases (HSV, VZV, *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 mumps virus IgM assay. Samples that were seronegative for mumps virus IgM antibodies and seropositive for the cross-reactant were used in the study. 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	Number of expected negative samples	LIAISON® positive or equivocal results
hCMV IgM antibodies	32	2
VZV IgM antibodies	5	0
Parvovirus B19 IgM antibodies	33	1
HSV IgM antibodies	5	0
Rubella virus IgM antibodies	8	0
EBV IgM antibodies	41	4
Measles virus IgM antibodies	5	0
Parainfluenza viruses antibodies	3	0
<i>Toxoplasma gondii</i> IgM antibodies	13	0
<i>Treponema pallidum</i> antibodies	4	0
Rheumatoid factor (anti-Fc immunoglobulin)	5	0
Anti-nuclear autoantibodies (ANA)	5	0
Human anti-mouse antibodies (HAMA)	7	0
Total	166	7

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	Positive control
Number of determinations	20	20	20
Mean (index value)	1.2	1.5	2.3
Standard deviation	0.07	0.05	0.05
Coefficient of variation (%)	5.9	3.5	3.1
Min. value (index value)	1.1	1.4	2.2
Max. value (index value)	1.3	1.5	2.4

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	Positive control
LOT No. 01			
Number of determinations	20	20	20
Mean (index value)	1.1	1.6	2.3
Standard deviation	0.07	0.08	0.13
Coefficient of variation (%)	6.2	5.3	5.8
Min. value (index value)	1.0	1.4	2.0
Max. value (index value)	1.2	1.7	2.5
LOT No. 02			
Number of determinations	20	20	20
Mean (index value)	1.1	1.5	2.6
Standard deviation	0.08	0.08	0.15
Coefficient of variation (%)	7.2	5.1	5.8
Min. value (index value)	1.0	1.4	2.3
Max. value (index value)	1.3	1.7	3.0
LOT No. 03			
Number of determinations	20	20	20
Mean (index value)	1.2	1.5	2.4
Standard deviation	0.08	0.09	0.17
Coefficient of variation (%)	6.4	5.7	7.0
Min. value (index value)	1.0	1.3	2.2
Max. value (index value)	1.3	1.7	3.0
Lot to lot coefficient of variation (%)	5.1	3.8	6.3

Reproducibility - Site 2	A	B	Positive control
LOT No. 01			
Number of determinations	15	15	15
Mean (index value)	1.3	1.7	2.2
Standard deviation	0.11	0.08	0.22
Coefficient of variation (%)	8.9	5.0	9.7
Min. value (index value)	1.0	1.5	1.9
Max. value (index value)	1.4	1.8	2.5
LOT No. 02			
Number of determinations	15	15	15
Mean (index value)	1.3	1.6	2.4
Standard deviation	0.09	0.08	0.17
Coefficient of variation (%)	7.1	5.0	7.0
Min. value (index value)	1.2	1.5	2.1
Max. value (index value)	1.5	1.7	2.7
LOT No. 03			
Number of determinations	15	15	15
Mean (index value)	1.4	1.7	2.2
Standard deviation	0.11	0.08	0.18
Coefficient of variation (%)	7.5	4.9	8.3
Min. value (index value)	1.3	1.6	1.9
Max. value (index value)	1.6	1.9	2.5
Lot to lot coefficient of variation (%)	4.3	3.5	5.1

15.3. Precision with LIAISON® XL 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 variability shown in the tables below did not result in sample misclassification.

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

Repeatability	1	2	Positive control
Number of determinations	20	20	20
Mean (index value)	1.3	1.6	2.3
Standard deviation	0.08	0.09	0.06
Coefficient of variation (%)	6.1	5.7	2.4
Min. value (index value)	1.2	1.4	2.2
Max. value (index value)	1.4	1.8	2.4

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

Reproducibility	1	2	Positive control
LOT No. 01			
Number of determinations	20	20	20
Mean (index value)	1.3	1.7	2.6
Standard deviation	0.08	0.08	0.15
Coefficient of variation (%)	6.1	4.6	5.7
Min. value (index value)	1.1	1.6	2.3
Max. value (index value)	1.4	1.8	2.8
LOT No. 02			
Number of determinations	20	20	20
Mean (index value)	1.0	1.4	2.3
Standard deviation	0.04	0.08	0.08
Coefficient of variation (%)	4.1	5.7	3.5
Min. value (index value)	0.9	1.3	2.2
Max. value (index value)	1.1	1.5	2.4
LOT No. 03			
Number of determinations	20	20	20
Mean (index value)	1.2	1.6	2.3
Standard deviation	0.07	0.06	0.12
Coefficient of variation (%)	6.0	4.0	5.2
Min. value (index value)	1.1	1.5	2.1
Max. value (index value)	1.4	1.7	2.5
Lot to lot coefficient of variation (%)	13.1	9.8	7.2

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® Mumps IgM Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of eight (8) frozen samples was used for the study.

The samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels.

The LIAISON® Control Mumps 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 mean Index value, 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. 8 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	3	4	5	6	7	8	9	10	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.71	0.70	0.75	1.4	2.2	2.4	2.8	2.5	2.3
Standard deviation	0.02	0.01	0.01	0.05	0.06	0.08	0.06	0.07	0.08
Coefficient of variation (%)	2.9	2.3	2.5	3.4	2.9	3.3	2.4	2.8	3.6
Min. value (index value)	0.59	0.62	0.67	1.3	1.9	2.2	2.4	2.1	1.9
Max. value (index value)	0.81	0.79	0.86	1.7	2.6	2.7	3.2	2.9	2.6

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 8 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	3	4	5	6	7	8	9	10	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (index value)	0.71	0.70	0.75	1.4	2.2	2.4	2.8	2.5	2.3
Standard deviation	0.03	0.03	0.04	0.07	0.10	0.11	0.11	0.12	0.13
Coefficient of variation (%)	5.1	5.3	5.4	5.3	4.6	4.5	4.1	4.7	6.0
Min. value (index value)	0.59	0.62	0.67	1.3	1.9	2.2	2.4	2.1	1.9
Max. value (index value)	0.81	0.79	0.86	1.7	2.6	2.7	3.2	2.9	2.6

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).

Four samples containing high concentrations of Mumps virus IgM were diluted in specimen diluent then were tested to evaluate saturation effect on the LIAISON® platform. 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 550 specimens collected from a European laboratory routine. The specimens were tested by a comparison method and consensus with additional clinical and serological data was applied to define the expected results. Seventeen specimens were unresolved by the reference method and therefore were not included in the data analysis.

One positive, one equivocal and 437 negative results were observed in the expected negative population studied - diagnostic specificity: 99.5% (437/439) (95% confidence interval: 98.3-99.9%).

Four negative, nine equivocal and 81 positive results were observed in the expected positive population studied - diagnostic sensitivity: 95.2% (81/85) (95% confidence interval: 88.3-98.7%).

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 Rubin, S., Eckhaus, M., Rennick, L. J., Bamford, C. G. & Duprex, W. P. Molecular biology, pathogenesis and pathology of mumps virus. *J Pathol* 235, 242-252, (2015).
- 2 White, S. J., Boldt, K. L., Holditch, S. J., Poland, G. A. & Jacobson, R. M. Measles, mumps, and rubella. *Clin Obstet Gynecol* 55, 550-559, (2012).
- 3 Cooley, L. (Public Health Learning Network - Australia, Australia, 2015).
- 4 Latner, D. R. & Hickman, C. J. Remembering mumps. *PLoS Pathog* 11, e1004791, (2015).
- 5 Alter, S. J., Bennett, J. S., Koranyi, K., Kreppel, A. & Simon, R. Common childhood viral infections. *Curr Probl Pediatr Adolesc Health Care* 45, 21-53, (2015).
- 6 Woo, T. M. Postexposure Management of Vaccine-Preventable Diseases. *J Pediatr Health Care* 30, 173-182; quiz 183-174, (2016).
- 7 Public Health Agency of Canada. Vol. 36S1 Canada Communicable Disease Report 1-46 (Canada, 2010).
- 8 Wilson, K. F., Meier, J. D. & Ward, P. D. Salivary gland disorders. *Am Fam Physician* 89, 882-888, (2014).
- 9 Chanal, J. et al. Lipschutz genital ulceration associated with mumps. *Dermatology* 221, 292-295, (2010).
- 10 Street, E., Joyce, A. & Wilson, J. BASHH UK guideline for the management of epididymo-orchitis, 2010. *Int J STD AIDS* 22, 361-365, (2011).
- 11 Ternavasio-de la Vega, H. G. et al. Mumps orchitis in the post-vaccine era (1967-2009): a single-center series of 67 patients and review of clinical outcome and trends. *Medicine (Baltimore)* 89, 96-116, (2010).
- 12 Watanabe, M. et al. Mumps virus-associated acute encephalopathy: case report and review of the literature. *J Child Neurol* 28, 243-245, (2013).
- 13 Solomon, I. H. & Milner, D. A., Jr. Histopathology of vaccine-preventable diseases. *Histopathology* 70, 109-122, (2017).
- 14 Bockelman, C., Frawley, T. C., Long, B. & Koyfman, A. Mumps: An Emergency Medicine-Focused Update. *J Emerg Med* 54, 207-214, (2018).
- 15 Clarkson, E., Mashkoor, F. & Abdulateef, S. Oral Viral Infections: Diagnosis and Management. *Dent Clin North Am* 61, 351-363, (2017).
- 16 Gadler, T., Martinez, N. & Ogg-Gress, J. Recognizing Measles, Mumps, and Rubella in the Emergency Department. *Adv Emerg Nurs J* 40, 110-118, (2018).
- 17 Kutty, P. K. et al. Guidance for isolation precautions for mumps in the United States: a review of the scientific basis for policy change. *Clin Infect Dis* 50, 1619-1628, (2010).
18. BOWEN et al., Impact of blood collection devices on clinical chemistry assay *Clinical Biochemistry*, 43, 4-25, 2010.
19. Clinical and Laboratory Standards Institute (CLSI) C15-A3, Vol.34, No.12, User Verification of Precision and Estimation of Bias; Approved Guideline - Third Edition

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