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, §9, §15.2, §15.3, §15.5, §15.7, §15.8, §15.9, References;
Deletions: §14;

LIAISON® Rubella IgM (REF 310730)

1. INTENDED PURPOSE

The LIAISON® Rubella IgM uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of IgM antibodies to rubella virus in human serum and plasma samples. The assay is intended for use as an aid in the determination of immune status to Rubella in individuals including pregnant women, and as an aid in the diagnosis of Rubella virus infection. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Rubella, one of the classic childhood exanthemas, is caused by rubella virus, a positive-sense, single-stranded RNA virus of the Togaviridae family[1].
While rubella virus infection usually causes a mild fever and rash illness in children and adults, infection during pregnancy, especially during the first trimester, can result in miscarriage, fetal death, stillbirth, or infants with congenital malformations, known as congenital rubella syndrome (CRS)[3].
CRS symptoms include, but are not limited to, fetal death, spontaneous abortion, premature delivery, ocular abnormalities (e.g., cataracts and microophthalmia), neurological problems (e.g., intellectual disability), abnormal cardiac development and, most commonly, deafness. Congenital malformations may be present at birth or sometimes develop months to years after birth. Examples of delayed CRS-induced maladies are type I diabetes mellitus, deafness, intellectual disability, subacute encephalitis. The frequency and severity of CRS decreases as gestation progresses. Maternal exposure to rubella during the first 12 weeks of pregnancy results in CRS in 85% of developing embryos/fetuses. Conversely, after the 20th gestational week, the risk of congenital defects is minimal. Nevertheless, neonatal rubella infections are possible when non-immune mothers transmit rubella to the foetus close to delivery.
In CRS, rubella virus is able to infect the placenta, spread to the fetus, and alter the function of multiple fetal systems by interfering with organ formation and causing systemic inflammation[1]. Since rubella vaccines were introduced in 1969, vaccination strategies have led to the elimination of rubella and CRS in many countries[2]. In order to prevent the CRS it is important to assess Rubella immunity in health workers and women of childbearing age and to screen all pregnant women, given the low coverage of Rubella vaccination (about 52%)[4].
Seronegative women of childbearing age and healthcare workers who need to be protected against rubella should continue to be offered rubella vaccine, usually as combined MMR (Measles, Mumps, Rubella) vaccine.
The first humoral immune response to infection is the synthesis of specific anti-rubella virus IgM antibody, which reaches high serum levels two weeks after the rash and stays in circulation for one to two months. The specific IgG antibody generally appears a few days after the onset of rash, about one week after IgM develops. It rapidly increases to reach a plateau six to ten weeks after the onset of symptoms and then progressively decreases to a level (15-200 IU/mL) lasting for the whole life. Reinfection, which is completely asymptomatic, is accompanied by moderately increased levels of specific IgG[2].
Correct detection of IgM and IgG antibodies to rubella virus provides an essential tool for diagnosing and following up acute infection, for the assessment of immune status in fertile women and, therefore, for adopting suitable prophylaxis in susceptible women of child-bearing age. Since a vaccine was made available, the assay of IgG to rubella virus has been widely used to determine seroconversion of the recipient after vaccination. In developed countries, women of childbearing age are routinely screened for rubella antibodies to identify and vaccinate susceptible women. Immunity to rubella is normally determined by measuring the Rubella IgG with immunoassays that provide results in international units (IU) per milliliter[2]. The WHO Expert Committee on Biological Standardization (WHO-ECBS) agreed in October 2017 that the first international standard for anti-Rubella IgG (RUBI-1-94) should continue to be made available as a well characterised reference material[5].

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgM to rubella virus is an antibody capture chemiluminescence immunoassay (CLIA). IgG to human IgM (mouse, monoclonal) is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody to rubella virus is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, IgM antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, rubella virus antigen reacts with IgM directed against rubella virus that are already bound to the solid phase. During the third incubation, the antibody conjugate reacts with the immune complex formed during the second incubation, thus revealing that the immunological reaction has taken place. After the first and third incubations, 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 is indicative of rubella virus IgM concentration present in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.3 mL)	SORB	Magnetic particles (≥0.25% solid) coated with IgG to human IgM (mouse, monoclonal) (approximately 0.200 mg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (1.5 mL)	CAL 1	Human serum/plasma containing low rubella virus IgM levels (approximately 20-35 AU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (1.5 mL)	CAL 2	Human serum/plasma containing high rubella virus IgM levels (approximately 70-250 AU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Antigen (2.3 mL)	Ag	Inactivated rubella viral particle (HPV 77 strain) (approximately 13 µg/mL), BSA, buffer, 0.2% ProClin™ 300, preservatives.
Specimen diluent (2 x 28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye.
Conjugate (5 mL)	CONJ	Mouse monoclonal antibodies to rubella virus conjugated to an isoluminol derivative (approximately >1µg/mL), non-specific IgG (mouse polyclonal), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives.
Number of tests		100

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

Additionally required materials

LIAISON® Rubella IgM controls (negative and positive) (REF 310731).

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.

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 DIL SPE, CONJ	Ag
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 Aquatic chronic 3 H412 Repr. 1B H360FD
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark	  GHS07 Exclamation mark GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. H360FD May damage fertility. May damage the unborn child.
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.	P261 Avoid breathing dust/fume/gas/mist/vapours/ spray. P280 Wear protective gloves/protective clothing/ eye protection/face protection. P363 Wash contaminated clothing before reuse. P202 Do not handle until all safety precautions have been red and understood. P308+P313 If exposed or concerned: get medical advice/attention.
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).	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); Boric Acid.

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:

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 and LIAISON® XS analyzers are equipped with 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. STORAGE AND STABILITY OF REAGENT INTEGRAL

Upon receipt, the reagent integral must be stored in an upright position to facilitate resuspension of magnetic particles. When the reagent integral is stored sealed and kept upright, the reagents are stable at 2-8°C up to the expiry date. Do not freeze. The reagent integral should not be used past the expiry date indicated on the kit and reagent integral labels. After removing the seals, the reagent integral is stable for eight weeks refrigerated either at 2-8°C or on board the instrument.

9. SPECIMEN COLLECTION AND PREPARATION

The correct specimen type must be used in the assay. Following matrices have been tested and may be used:
- serum,
- potassium EDTA,
- citrate plasma.

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:
- the 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 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 calibrations to be performed.

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 weeks before.
- **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 Analyzer: Calibrator values are stored in the reagent integral 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

This test requires the following assay files: Rub-Mc, Rub-M and RM-cAg.

To test specimens use Rub-M or Rub-Mc.

Never use RM-cAg.

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.

Assay file for rubella IgM determination is Rub-M.

The analyzer operations are as follows:

1. Dispense specimen diluent and coated magnetic particles.
2. Dispense calibrators, controls or specimens into the reaction module.
3. Incubate.
4. Wash with Wash/System liquid.
5. Dispense specimen diluent and antigen into the reaction module.
6. Incubate.
7. Dispense tracer into the reaction module.
8. Incubate.
9. Wash with Wash/System liquid.
10. Add the Starter Kit and measure the light emitted.

An additional assay procedure (see section 16) may be performed with no rubella virus antigen added in a given rubella virus IgM-equivocal or IgM-positive sample to check for reaction specificity to rubella virus antigen. The procedure allows the measured concentration of a sample non-added with rubella virus antigen (i.e., mimicking a non-specific reaction) to be compared with the measured concentration of the same sample added with rubella virus antigen (i.e., following the standard assay procedure).

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Rubella IgM controls
(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 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 rubella virus IgM antibody concentrations expressed as AU/mL 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. 10 to 400 AU/mL rubella virus IgM.
Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:10). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows up to 40 sample predilutions to be performed. **Warning** - *Refer to §15.4 for dilution test*.

Sample results should be interpreted as follows:
Samples with rubella virus IgM concentrations below 20 AU/mL should be graded *negative*.
Samples with rubella virus IgM concentrations ranging between 20 and 25 AU/mL should be graded *equivocal*.
Samples with rubella virus IgM concentrations equal to or above 25 AU/mL should be graded *positive*.

A positive result is generally indicative of acute infection. A negative result, however, does not always rule out acute rubella, because the infection is in its very early stage and the patient may be still unable to synthesize rubella virus specific IgM. If clinical exposure to rubella 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 rubella virus IgM. Serological data from detection of additional rubella virus markers may provide useful information for clinical interpretation of results.

Test results are reported quantitatively as positive or negative for the presence of rubella 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.

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.

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, EDTA), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), or by freeze-thaw cycles of samples. Heparinized plasma specimens interfere with assay results and may not be used.

Cross-reactions. The cross-reactivity study for the LIAISON® Rubella IgM assay was designed to evaluate potential interference from antibodies to other organisms that may cause infectious diseases (hCMV, parvovirus B19, *Toxoplasma gondii*, EBV, HBV, HAV, HSV, hHV 6, *Treponema pallidum*, VZV, measles virus, mumps virus, *Borrelia burgdorferi*, influenza viruses) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor). Samples for these studies were pre-screened with another commercially available rubella IgM assay. If found negative for rubella 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.

Condition	Number of expected negative samples	LIAISON® positive and equivocal results
Parvovirus B19 IgM antibodies	56	0
Toxoplasma gondii IgM antibodies	13	0
EBV IgM antibodies	34	0
hCMV IgM antibodies	26	0
HAV IgM antibodies	60	0
HBc IgM antibodies	10	0
HSV-1/2 IgM antibodies	11	0
Influenza viruses antibodies	8	0
Borrelia burgdorferi IgM antibodies	9	0
Mumps virus IgM antibodies	10	0
Measles virus IgM antibodies	3	1 +
VZV IgM antibodies	16	0
hHV 6 antibodies	2	0
Treponema pallidum IgM antibodies	3	0
Anti-nuclear autoantibodies (ANA/ENA)	44	3 +
Rheumatoid factor (anti-Fc immunoglobulin)	60	0
Total	365	4 +

The combined use of rubella serological markers and clinical data is recommended when the diagnosis of acute rubella infection is based on a single specimen. A single result should not be used for diagnosis.

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

Repeatability on LIAISON® instrument.

Repeatability	Sample A	Sample B	Sample C
Number of determinations	20	20	20
Mean (AU/mL)	32.7	72.6	97.5
Standard deviation	2.79	4.31	7.86
Coefficient of variation (%)	8.5	5.9	8.1
Min. value	26.7	61.8	80.9
Max. value	39.3	79.7	109.3

Reproducibility on LIAISON® instrument.

Reproducibility	Sample A	Sample B	Sample C
Number of determinations	20	20	20
Mean (AU/mL)	31.7	68.9	91.3
Standard deviation	4.16	7.23	10.92
Coefficient of variation (%)	13.1	10.5	12.0
Min. value	23.5	60.0	71.3
Max. value	41.3	82.2	109.1

15.3. Inter-Lot Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine Inter-lot 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.

Inter-Lot Precision	D	E	F	G	H	I	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (AU/mL)	17.3	54.4	295	16.8	39.5	197	2.29	55.5
Standard deviation	2.266	3.972	24.58	1.531	2.302	16.73	0.445	6.32
Coefficient of variation (%)	12	5	6	0	1	8	14	9
Min. value (AU/mL)	14.0	46.7	236	14.8	35.5	155	1.56	48.0
Max. value (AU/mL)	22.2	61.8	336	19.4	43.8	219	3.16	67.8

15.4. 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 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	5	4	6	3	8	10	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (AU/mL)	29.6	40.9	44.2	56.9	57.4	92.0	150	1.95	75.4
Standard deviation	1.92	2.64	2.24	2.16	1.92	4.16	10.08	0.48	2.88
Coefficient of variation (%)	6.5	6.5	5.1	3.8	3.4	4.5	6.7	24.6	3.8
Min. value	26.4	37.3	40.7	52.6	53.0	82.8	131	1.32	70.7
Max. value	32.3	49.7	47.9	60.4	62.2	102	166	3.16	82.0

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

Reproducibility	1	2	5	3	7	8	9	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (AU/mL)	25.2	33.2	35.1	50.4	77.2	79.9	134	1.49	74.0
Standard deviation	2.44	3.41	3.12	4.76	5.62	6.06	12.88	0.51	6.09
Coefficient of variation (%)	9.7	10.3	8.9	9.4	7.3	7.6	9.6	34.0	8.2
Min. value	20.9	28.2	29.3	40.2	65.7	68.5	108	0.425	64.1
Max. value	30.0	39.0	43.6	59.3	87.3	88.0	160	2.58	82.9

15.5. Precision with LIAISON® XS Analyzer

A five day precision study was conducted on three LIAISON® XS analyzers to verify the precision with the LIAISON® Rubella 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 samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels.

The LIAISON® Control Rubella 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 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. Seven (7) serum samples containing different concentration of analyte and kit controls were assayed in six (6) replicates per day, over five (5) operating days, on three (3) units and one reagent lot.

Repeatability	10	11	12	13	14	15	16	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (AU/mL)	20.7	37.3	46.0	27.5	60.2	111	148	2368	72.4
Standard deviation	1.65	2.09	2.93	1.39	4.37	8.35	8.45	96.4	3.78
Coefficient of variation (%)	8.0	5.6	6.4	5.0	7.3	7.5	5.7	4.1	5.2
Min. value (AU/mL)	10.6	22.5	16.6	20.3	42.7	69.6	107	1926	53.8
Max. value (AU/mL)	24.2	42.8	51.9	32.1	81.2	163	165	2623	84.2

*Negative Control is expressed in RLU because out of the Assay Range.

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

Reproducibility	10	11	12	13	14	15	16	Negative control*	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (AU/mL)	20.7	37.3	46.0	27.5	60.2	111	148	2368	72.4
Standard deviation	2.29	3.35	4.18	2.01	6.02	11.7	9.97	109	4.88
Coefficient of variation (%)	11.1	9.0	9.1	7.3	10.0	10.6	6.7	4.6	6.7
Min. value	10.6	22.5	16.6	20.3	42.7	69.6	107	1926	53.8
Max. value	24.2	42.8	51.9	32.1	81.2	163	165	2623	84.2

*Negative Control is expressed in RLU because out of the Assay Range.

15.6. 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 one high-titred sample positive for rubella virus IgM. This sample resulted in a concentration value above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.7. Analytical and Functional Sensitivity

The analytical sensitivity was determined following the method from CLSI EP17-A) as Detection Capability (LoB, LoD, LoQ), reported below for each of the three platforms.

LIAISON® Analyzer
- Limit of Detection (LoD): 7.78 AU/mL.
- Limit of Blank (LoB)*: ≤ 5.51 AU/mL.
- Limit of Quantitation (LoQ): 6.45 AU/mL.

LIAISON® XL Analyzer
- Limit of Detection (LoD): 6.55 AU/mL.
- Limit of Blank (LoB)*: ≤ 4.48 AU/mL.
- Limit of Quantitation (LoQ): 6.04 AU/mL.

LIAISON® XS Analyzer
- Limit of Detection (LoD): 5.12 AU/mL.
- Limit of Blank (LoB)*: ≤ 3.43 AU/mL.
- Limit of Quantitation (LoQ): 4.68 AU/mL.

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

15.8. Trueness

Trueness is defined as the closeness of agreement between the average value obtained from a large series of test results and an accepted reference value (EP9-A3). On platform LIAISON® XL the recovery is within the acceptance criteria of 80% - 120% for all sample except for sample RUBM-S2 that shows a recovery 77.7%, but the assay performance in terms of trueness is considerate adequate since the average recovery is 84.5% therefore within acceptance criteria and the linear regression shows a slope = 0.998 and R = 0.992 (R2= 0.984).

Sample		Low (%)	High (%)	Expected (AU/mL)	Measured (AU/mL)	Recovery (%)
toto NS	**RUBM-S1**	100	-	14.2	14.2	-
2 NS + 1 PS	**RUBM-S2**	66.7	33.3	84.7	65.8	77.7
1 NS + 1 PS	**RUBM-S3**	50	50	121	108	89.2
1 NS + 1 PS	**RUBM-S4**	33.3	66.7	157	136	86.7
toto PS	**RUBM-S5**	-	100	228	228	-
				Average Recovery		**84.5**

15.9. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 1662 specimens from different selected populations (subjects never infected by rubella virus, subjects affected by autoimmune diseases, patients affected by various infectious diseases with similar symptomatology, subjects with past rubella infection or vaccine recipients, patients affected by acute rubella infection or re-infection, subjects with long-lasting rubella virus IgM and pregnant women). The specimens were tested by several comparison methods and consensus between them as well as the available clinical and serological data were applied to define the expected results. 76 specimens were unresolved by the reference methods and therefore were not included in the data analysis.

11 positive, 12 equivocal (reactive) and 1389 negative results were observed in the expected negative population studied - diagnostic specificity: 1389/1412, i.e. 98.37% (95% confidence interval: 97.57-98.96%).

2 negative, 15 equivocal (reactive) and 157 positive results were observed in the expected positive population studied - diagnostic sensitivity: 172/174, i.e. 98.85% (95% confidence interval: 95.91-99.86%).

16. ASSAY PROCEDURE WITH NO ANTIGEN ADDED

An additional assay procedure may be performed with no rubella virus antigen added in a given rubella virus IgM-equivocal or IgM-positive sample to check for reaction specificity to rubella virus antigen. The procedure allows the measured concentration of a sample non-added with rubella virus antigen (i.e., mimicking a non-specific reaction) to be compared with the measured concentration of the same sample added with rubella virus antigen (i.e., following the standard assay procedure). The procedure is the same as the standard assay procedure described in section 11, except for the fact that no rubella virus antigen is added. It must be performed with the same calibration and within the same working day as the standard assay procedure by selecting assay file Rub-Mc.

Interpretation of results

The analyzer automatically calculates the index value for specific IgM to rubella virus (ratio of a non-added specimen to a rubella antigen-added specimen) 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.

Assay range. 0.01 to 0.95 index for rubella virus IgM.

Sample results should be interpreted as follows:
An index value for rubella virus IgM below 0.300 generally indicates the presence of a specific reaction to LIAISON® rubella virus antigen.
An index value for rubella virus IgM equal to or above 0.300 generally indicates the presence of a non-specific reaction.
Samples with index value greater than 0.95 should be retested. If the index value is confirmed, the result should be graded as indicated for index value above 0.300.

All 168 expected positive samples for rubella virus IgM collected during the kit performance evaluation were tested for reaction specificity to rubella virus antigen and gave index values below 0.300.
15 samples expected to be negative for rubella virus IgM, but found either equivocal or positive by LIAISON® Rubella IgM assay were tested for reaction specificity to rubella virus antigen: 12 samples gave index values above 0.300 and 3 gave index values below 0.300.

Diagnostic specificity of the LIAISON® Rubella IgM assay after performing the procedure with no antigen added can be estimated on the basis of the results observed during performance evaluation. Out of 23 specimens expected to be negative for rubella virus IgM, but found either equivocal or positive by LIAISON® Rubella IgM assay, 18 samples can be expected to give index values above 0.300 and 5 samples can be expected to give index values below 0.300. On this assumption diagnostic specificity of the LIAISON® Rubella IgM assay was estimated as 1407/1412, i.e. 99.65% (95% confidence interval: 99.16-99.89%).

A Summary of safety and performance is available on EUDAMED.

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. Lambert N, Strebel P, Orenstein W, Icenogle J, Poland GA.
Rubella.
Lancet 2015;385:2297—307.

2. Bouthry E, Picone O, Hamdi G, Grangeot-Keros L, Ayoubi JM, Vauloup-Fellous C.
Rubella and pregnancy: diagnosis, management and outcomes.
Prenat Diagn. 2014 Dec; 34(13):1246-53. dci: 10.1002/pd.4467. Epub 2014 Sep 16. Review.

3. Grant GB, Reef SE, Patel M, Knapp JK, Dabbagh A.
Progress in Rubella and Congenital Rubella Syndrome Control and Elimination - Worldwide, 2000-2016.
MMWR Morb Mortal Wkly Rep. 2017 Nov 17;66(45): 1256-1260. doi: 10.15585/mmwr.mm6645a4.

4. WHO Expert Committee on Biological Standardization: sixty-eigth report. 2018
http :1/a pps.who. i nt/i ris/bitstream/hand le/ 10665/272807/9789241210201eng.pdf?ua=1&ua=1 (accessed Aug 9, 2019).

5. WHO. Global and regional immunization
profi le. 2018. http ://www.who. i nt/immunization/monitori ng_survei l lance/data/gs_gloprofile.pdf?ua=1 (accessed Jan 19, 2018)

6. BOWEN et al
Impact on blood collection devices on clinical chemistry assay
Clinical Biochemistry , 43, 4-25, 2010

200/007-829, 11 - 2024-01