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Deletions: -

LIAISON® Rubella IgG II (REF 317260)

1. INTENDED PURPOSE

The LIAISON® Rubella IgG II assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination and qualitative detection of specific IgG antibodies to rubella virus in human serum or plasma samples. The assay is intended as screening to determine the immunological status of individuals, including previously vaccinated subjects and 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, by 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]. Given the actual world coverage Rubella vaccination (about 52%)[4] in order to prevent the CRS it is important to assess rubella immunity in health workers and in women of childbearing age and to screen all pregnant women to determine their rubella serostatus.

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 and qualitative detection of specific IgG to rubella virus is an indirect chemiluminescence immunoassay (CLIA). Rubella antigen is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, rubella virus antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with rubella virus IgG 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 is indicative of rubella virus IgG concentration present in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

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

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with inactivated rubella viral particle (HPV 77 strain) (approx. 100 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.7 mL)	CAL 1	Human serum/plasma containing low rubella virus IgG levels (approx. 9.85 IU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (IU/mL) are referenced to RUBI–1–94 – NIBSC 1st International Standard for anti-Rubella Immunoglobulin, Human (1997).
Calibrator 2 (2.7 mL)	CAL 2	Human serum/plasma containing high rubella virus IgG levels (approx. 280 IU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (IU/mL) are referenced to RUBI–1–94 – NIBSC 1st International Standard for anti-Rubella Immunoglobulin, Human (1997).
Specimen diluent (2 x 28 mL)	DIL SPE	BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives.
Number of tests		100

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® 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® Rubella IgG II controls (negative and positive) (REF 317261).

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 and anti-HIV-2 and were 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 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. Waste must be handled with care and disposed of in compliance with the laboratory guidelines and with 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, DIL SPE, 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 the Safety Data Sheets available on www.diasorin.com.

7. PREPARATION OF THE 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 in the magnetic particle compartment until the color 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 are 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 use. 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 use. 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 THE REAGENT INTEGRAL

- **Sealed**: stable at 2-8°C until the expiry date.
- **Opened on board or at 2-8°C**: up to twelve (12) weeks.
- Use the storage rack provided with the LIAISON® Analyzer family for upright storage of the reagent integral.
- Do not freeze.
- Keep upright for storage to facilitate subsequent proper resuspension of the magnetic particles.
- Keep away from direct light.

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;
– plasma collected with the following anticoagulant:
  .lithium heparin;
  .sodium heparin;
  .K2-EDTA.

The use of citrated plasmas causes underestimation of positive results.

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 for 48 hours, in any case, room temperature storage should be avoided;
– 2°-8°C for 8 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 (4) 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 eight (8) 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 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

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 analyzer cannot read the barcode label, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instructions.

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 analyzer cannot read the RFID Tag, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instructions.

The analyzer operations are as follows:
1. Dispense diluent and coated magnetic particles into the reaction module.
2. Dispense calibrators, controls or specimens.
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.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Rubella IgG II controls (REF 317261)

(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 in agreement with the 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 IgG antibody concentrations expressed as IU/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. 0.2 to 350 IU/mL Rubella virus IgG.

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 20 sample predilutions to be performed.

Sample results should be interpreted as follows:
Samples with rubella virus IgG concentrations equal to or above 10 IU/mL should be graded *positive*.
Samples with rubella virus IgG concentrations ranging between 7 and 10 IU/mL should be graded *equivocal*.
Samples with rubella virus IgG concentrations below 7 IU/mL should be graded *negative*.

A positive result generally indicates exposure to the pathogen, either through vaccination or prior infection.

An equivocal result should be interpreted with care, as it may indicate a low level of IgG antibodies to rubella virus in the sample.

A negative result may indicate absence or very low level of IgG antibodies to rubella virus in the sample.

A negative result does not rule out the possibility of an acute infection. The test could score negative in infected patients during the incubation period and the early stages of infection. If exposure to rubella virus is suspected despite a negative finding, a second sample should be collected and tested within one or two weeks at the latest. Seroconversion from a negative sample to a positive sample is evidence of either recent infection, or response to vaccination, or administration of immunoglobulins.

A level of IgG antibodies lower than 10 IU/mL may be obtained from patients with a recent infection, but also from patients exposed to the virus in the past or vaccinated, which have had a weak or transient antibody response, or the decline in antibody titers in the period after contact. Therefore, recent infections could be identified by assessing subsequent samples collected and tested with a two- to three-week delay. Subsequent samples should be assessed in the same run. In this case, if the antibody levels are rising, a recent infection may be suspected. Serological data from detection of additional rubella virus markers or from alternative methods, as well as information regarding the patient's vaccination history, 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 quantitatively as positive, equivocal or negative for the presence of rubella virus IgG. 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, patient history, vaccination records, other diagnostic procedures, and always in association with medical judgment.
- The level of protection in patients, especially those with low titers of rubella virus IgG, should be determined by a physician.
- 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 (for details, refer to §15.1).
- Results obtained with LIAISON® Rubella IgG II assay may not be used interchangeably with values obtained with different manufacturers' assay methods.
- 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 analytes in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, hemolysis, 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 (EDTA, sodium and lithium heparin), hemolysis (up to 1000 mg/dL hemoglobin), lipemia (up to 3000 mg/dL triglycerides), bilirubinemia (up to 20 mg/dL bilirubin), or by freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® Rubella IgG II assay was designed to evaluate potential interference from antibodies to other organisms that may cause infectious diseases (EBV, CMV, parvovirus B19, Toxoplasma, HSV, measles and mumps viruses, Mycoplasma p. and VZV) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies (ANA), anti-mouse human antibodies (HAMA) and rheumatoid factor (anti-Fc immunoglobulin)). Samples for these studies were pre-screened with another commercially available Rubella IgG assay. If found negative for Rubella IgG 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.

Autoimmunity/Infectivity Marker (samples positive for antibodies to)	No. of samples	Number of reactive samples with Rubella IgG II (>7 IU/mL)
CMV IgG	12	0
EBV IgG (EBNA IgG and VCA IgG)	12	0
HSV 1/2 IgG	12	0
Measles IgG	12	0
Mumps IgG	12	0
Mycoplasma p. IgG	12	0
Parvovirus IgG	12	0
Toxoplasma IgG	12	0
VZV IgG	12	0
ANA	8	0
RF	6	0
HAMA	8	0
TOTAL	130	0

None of the 130 specimens tested from the disease panel was positive. There was no conclusive evidence of cross-reactivity observed; however, due to the limited availability of certain samples, the possibility of cross-reactivity cannot be excluded. The results refer to the groups of samples investigated and are not guaranteed specifications, as differences may exist between laboratories and locations.

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analytes, 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. The results refer to the groups of samples investigated and are not guaranteed specification, as differences may exist between laboratories and locations.

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

Repeatability Lot #01	A	B	C	D	E	F	G	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.7	8.9	17.5	11.4	37.2	198	135	< 0.2	24.5
Min (IU/mL)	2.2	8.0	16.0	10.1	33.5	173	119	< 0.2	21.3
Max (IU/mL)	2.9	9.8	18.5	13.5	39.4	216	147	< 0.2	26.2
Standard Deviation	0.18	0.43	0.69	0.86	1.42	13.2	8.5	n.a.	1.2
Coefficient of variation (%)	6.7%	4.8%	3.9%	7.6%	3.8%	6.7%	6.3%	n.a.	4.9%

Reproducibility. Twenty replicates were performed on different days (maximum of two runs per day) on three integral lots 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 Lot #01 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	3.0	8.2	18.8	12.5	43.2	233	< 0.2	28.9
Min (IU/mL)	2.2	6.6	15.8	11.3	38.1	189	< 0.2	25.6
Max (IU/mL)	3.8	9.7	21.0	14.2	50.5	269	< 0.2	33.1
Standard Deviation	0.4	0.77	1.8	1.0	3.5	24.0	n.a.	2.37
Coefficient of variation (%)	14.0%	9.4%	9.7%	7.7%	8.1%	10.3%	n.a.	8.2%

Reproducibility Lot #02 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.4	8.9	17.2	11.5	40.2	234	< 0.2	26.7
Min (IU/mL)	1.8	7.8	15.4	9.8	34.7	181	< 0.2	22.6
Max (IU/mL)	3.1	10.0	19.7	12.9	45.0	279	< 0.2	29.9
Standard Deviation	0.3	0.59	1.2	0.8	3.0	24.1	n.a.	2.18
Coefficient of variation (%)	13.7%	6.7%	6.8%	6.9%	7.4%	10.3%	n.a.	8.2%

Reproducibility Lot #03 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.9	8.4	19.3	12.3	40.8	222	< 0.2	28.2
Min (IU/mL)	2.4	7.3	16.6	10.7	35.5	184	< 0.2	24.7
Max (IU/mL)	3.6	9.6	22.2	15.1	50.9	251	< 0.2	30.7
Standard Deviation	0.4	0.67	1.5	1.0	4.1	18.9	n.a.	1.64
Coefficient of variation (%)	13.1%	8.0%	8.0%	8.2%	10.0%	8.4%	n.a.	5.8%

Reproducibility	LIAISON® Rubella IgG II (Code 317260), Site 1 on LIAISON®						
Sample ID	A	B	C	D	E	F	Positive control
Mean (IU/mL)	2.73	8.49	18.4	12.1	41.4	229	27.9
Inter-lot coefficient of variation (%)	12.0	4.30	5.66	4.09	3.56	2.12	3.93

Reproducibility Lot #01 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.5	8.2	18.2	12.0	39.0	192	< 0.2	26.8
Min (IU/mL)	2.0	6.9	16.0	10.7	33.9	145	< 0.2	24.4
Max (IU/mL)	2.9	9.6	20.4	13.2	44.3	248	< 0.2	29.8
Standard Deviation	0.2	0.78	1.1	0.7	2.5	27.1	n.a.	1.3
Coefficient of variation (%)	8.7%	9.5%	6.2%	5.9%	6.4%	14.1%	n.a.	4.9%

** test performed on another instrument at site 1*

Reproducibility Lot #02 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.5	9.2	18.8	12.3	40.1	209	< 0.2	27.5
Min (IU/mL)	1.8	7.9	14.5	10.1	32.3	174	< 0.2	22.2
Max (IU/mL)	3.0	10.8	20.9	13.9	46.4	252	< 0.2	30.7
Standard Deviation	0.2	0.79	1.3	0.9	2.7	20.3	n.a.	1.8
Coefficient of variation (%)	9.3%	8.5%	7.0%	7.1%	6.7%	9.7%	n.a.	6.7%

** test performed on another instrument at site 1*

Reproducibility Lot #03 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	3.0	7.8	19.6	12.7	40.6	180	< 0.2	27.9
Min (IU/mL)	2.7	6.6	14.9	11.9	37.2	140	< 0.2	26.0
Max (IU/mL)	3.3	9.0	22.0	13.7	44.3	220	< 0.2	30.5
Standard Deviation	0.2	0.71	1.4	0.5	1.7	22.6	n.a.	1.3
Coefficient of variation (%)	5.6%	9.2%	7.1%	3.8%	4.1%	12.6%	n.a.	4.7%

** test performed on another instrument at site 1*

Reproducibility	LIAISON® Rubella IgG II (Code 317260), Site 2 on LIAISON®						
Sample ID	A	B	C	D	E	F	Positive control
Mean (IU/mL)	2.66	8.42	18.8	12.3	39.9	193	27.4
Inter-lot coefficient of variation (%)	9.52	8.99	3.49	2.66	1.80	7.45	1.83

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analytes, 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. The results refer to the groups of samples investigated and are not guaranteed specification as differences may exist between laboratories and locations.

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

Repeatability Lot #01	A	B	C	D	E	F	G	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.1	9.0	17.0	11.7	39.1	209	129	< 0.2	26.3
Min (IU/mL)	1.9	8.4	16.2	10.6	36.8	200	122	< 0.2	25.3
Max (IU/mL)	2.4	9.2	17.9	12.5	41.9	221	140	< 0.2	27.3
Standard Deviation	0.14	0.20	0.48	0.44	1.21	7.1	4.5	n.a.	0.61
Coefficient of variation (%)	6.7%	2.2%	2.8%	3.8%	3.1%	3.4%	3.5%	n.a.	2.3%

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

Reproducibility Lot #01 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.2	9.1	17.3	11.2	39.8	203	< 0.2	26.5
Min (IU/mL)	1.9	7.7	16.0	10.1	36.0	179	< 0.2	24.6
Max (IU/mL)	2.6	9.7	19.0	12.3	45.5	238	< 0.2	28.8
Standard Deviation	0.2	0.54	0.9	0.6	2.6	18.2	n.a.	1.2
Coefficient of variation (%)	9.1%	5.9%	5.4%	5.5%	6.6%	9.0%	n.a.	4.5%

Reproducibility Lot #02 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.6	8.7	17.5	11.9	41.3	231	< 0.2	26.9
Min (IU/mL)	2.2	7.2	16.6	10.7	37.8	204	< 0.2	24.9
Max (IU/mL)	3.4	9.3	20.2	13.5	46.4	271	< 0.2	29.4
Standard Deviation	0.29	0.62	0.9	0.8	2.3	20.9	n.a.	1.2
Coefficient of variation (%)	13.1%	7.1%	8.0%	8.2%	10.0%	8.5%	n.a.	4.5%

Reproducibility Lot #03 site 1	A	B	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.9	8.8	19.0	12.9	43.8	235	< 0.2	28.8
Min (IU/mL)	2.6	7.2	17.1	10.7	39.0	195	< 0.2	25.4
Max (IU/mL)	3.5	9.7	21.7	14.9	48.7	269	< 0.2	33.6
Standard Deviation	0.3	0.66	1.3	1.1	2.9	19.1	n.a.	2.2
Coefficient of variation (%)	9.3%	7.5%	6.8%	8.5%	6.7%	8.1%	n.a.	7.7%

Reproducibility	LIAISON® Rubella IgG II (Code 317260), Site 1 on LIAISON® XL						
Sample ID	A	B	C	D	E	F	Positive control
Mean (IU/mL)	2.59	8.87	18.0	12.0	41.6	223	27.4
Inter-lot coefficient of variation (%)	13.8	2.04	5.00	7.08	4.81	7.62	4.38

Reproducibility Lot #01 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.6	8.1	19.0	12.8	42.0	191	< 0.2	27.8
Min (IU/mL)	2.1	6.9	17.7	11.0	38.0	158	< 0.2	25.9
Max (IU/mL)	2.9	9.1	20.4	14.6	44.8	219	< 0.2	29.8
Standard Deviation	0.2	0.68	0.8	0.8	1.7	13.6	n.a.	1.0
Coefficient of variation (%)	8.1%	8.5%	4.3%	6.5%	4.1%	7.1%	n.a.	3.7%

** test performed on another instrument at site 1*

Reproducibility Lot #02 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.6	8.5	17.8	11.9	40.4	202	< 0.2	26.3
Min (IU/mL)	2.2	7.3	16.7	10.7	38.3	180	< 0.2	24.8
Max (IU/mL)	3.1	10.0	20.0	13.1	42.6	241	< 0.2	29.1
Standard Deviation	0.2	0.7	0.8	0.6	1.4	16.3	n.a.	1.2
Coefficient of variation (%)	9.5%	7.8%	4.6%	5.4%	3.5%	8.1%	n.a.	4.4%

** test performed on another instrument at site 1*

Reproducibility Lot #03 site 2	A	B*	C	D	E	F	Negative control	Positive control
Number of determinations	20	20	20	20	20	20	20	20
Mean (IU/mL)	2.6	8.3	18.2	12.2	39.4	179	< 0.2	26.5
Min (IU/mL)	2.1	7.0	16.8	10.5	35.7	160	< 0.2	23.8
Max (IU/mL)	3.4	9.6	20.1	13.7	42.5	207	< 0.2	30.3
Standard Deviation	0.3	0.74	1.0	0.9	1.9	10.5	n.a.	1.7
Coefficient of variation (%)	13.1%	8.8%	5.4%	7.4%	4.7%	5.9%	n.a.	6.4%

** test performed on another instrument at site 1*

Reproducibility	LIAISON® Rubella IgG II (Code 317260), Site 2 on LIAISON® XL						
Sample ID	A	B	C	D	E	F	Positive control
Mean (IU/mL)	2.57	8.31	18.3	12.3	40.6	191	26.9
Inter-lot coefficient of variation (%)	0	2.45	3.23	3.97	3.13	5.87	2.92

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® Rubella IgG II Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 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 IgG II 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. 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	1	2	3	4	5	6	7	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (IU/mL)	3.58	12.7	37.6	40.3	82.4	102	192	1159*	28.0
Standard deviation	0.08	0.35	0.93	0.76	1.94	2.59	9.77	40.2	0.54
Coefficient of variation (%)	2.3	2.7	2.5	1.9	2.4	2.5	5.1	3.5	1.9
Min. value (IU/mL)	3.25	11.3	33.5	35.2	71.2	86.4	145	972	24.1
Max. value (IU/mL)	3.88	13.7	40.9	44.1	89.9	111	220	1361	32.7

*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. 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	1	2	3	4	5	6	7	Negative control	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90
Mean (IU/mL)	3.58	12.7	37.6	40.3	82.4	102	192	1159*	28.0
Standard deviation	0.13	0.46	1.41	1.58	3.31	4.66	14.0	78.9	2.09
Coefficient of variation (%)	3.5	3.6	3.8	3.9	4.0	4.6	7.3	6.8	7.5
Min. value (IU/mL)	3.25	11.3	33.5	35.2	71.2	86.4	145	972	24.1
Max. value (IU/mL)	3.88	13.7	40.9	44.1	89.9	111	220	1361	32.7

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

15.5. Linearity and Trueness

The assay linearity has been checked by the dilution test.

Dilution test. Four serum samples containing high rubella virus IgG concentrations were tested as such and after serially diluting with the specimen diluent. Rubella virus IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) were all above 0.99.

Dilution	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery	Dilution	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery
neat	–	179	–	neat	–	191	–
1:2	89.5	90.5	101	1:2	95.5	96	101
1:4	44.8	47.2	105	1:4	47.8	51.3	107
1:8	22.4	25.0	112	1:8	23.9	26.7	112
1:16	11.2	12.2	109	1:16	11.9	13.0	109
1:32	5.6	5.0	89	1:32	6.0	5.4	90

Dilution	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery	Dilution	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery
neat	–	>350	–	neat	–	>350	–
1:2	–	333	–	1:2.5	–	>350	–
1:4	166.5	151	91	1:5	–	180	–
1:8	83.3	75.9	91	1:10	90.0	92.9	103
1:16	41.6	39.6	95	1:20	45.0	48.6	108
1:32	20.8	20.9	100	1:40	22.5	26.0	116
1:64	10.4	9.8	94	1:80	11.3	13.0	116
1:128	5.2	4.4	85	1:160	5.6	5.5	97

The assay trueness has been checked by dilution test of WHO standard (WHO RUB-1-94)

Dilution	Expected concentration (IU/mL)	Measured concentration (IU/mL)	Recovery (%)
neat	-	> 350	-
1:2.5	-	> 350	-
1:5	200	180	90
1:10	100	92.9	93
1:20	50	48.6	97
1:40	25	26	104
1:80	12.5	13	104
1:160	6.25	5.45	87

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 the saturation effect was evaluated by testing five high-titred samples positive for rubella virus IgG. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification up to 1000 IU/mL.

15.7. Analytical and functional sensitivity

Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.649 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Rubella IgG II assay is 0.2 IU/mL.
Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® Rubella IgG II assay is 0.73 IU/mL.

15.8. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 1,548 specimens sent to the diagnostic laboratory for Rubella IgG testing. Specimens were collected from two different European laboratories. An additional population of 200 healthy subjects was included in the study. The specimens were tested by several comparison methods. Consensus between them and a blot confirmatory test as well as the available clinical and serological data were applied to define the expected results.

19 specimens were unresolved (even after resolution) and therefore not included in the data analysis.

1 positive and 114 negative results were observed in the expected negative population studied - diagnostic specificity: 99.13% (95% confidence interval: 95.25-99.98%). 1582 positive results, 24 equivocal results and 8 negative results were observed in the expected positive population studied - diagnostic sensitivity: 99.50% (95% confidence interval: 99.03-99.78%), considering equivocal results as reactive.

Among above tested population, sent to laboratory for Rubella IgG testing, 303 were pregnant women. The specimens were tested by several comparison methods. Consensus between them and a blot confirmatory test as well as the available clinical and serological data were applied to define the expected results.

20 samples were observed in the expected negative population. Due to low number of negative samples, the diagnostic specificity will be calculated in combination with the overall population. 279 positive and 4 negative results were observed in the expected positive population studied - diagnostic sensitivity: 98.59% (279/283) (95% CI = 96.42 - 99.61%) considering equivocal results as reactive.

A different study was performed by testing 119 selected specimens not reactive for rubella virus IgG (Biomex panel NP-RUB-001 and a selected population from a clinical site): 119 negative results (below 7 IU/mL) were observed by using the LIAISON® Rubella IgG II assay. The diagnostic specificity is 100% (95% confidence interval: 96.95-100%).

A population of 99 subjects who have undergone vaccination was evaluated and 99 positive results were obtained – diagnostic sensitivity 100% (95% confidence interval: 96.34-100%).

Taking into consideration all populations and selected panels, the following diagnostic specificity and sensitivity were found:

– Overall diagnostic specificity: 99.57% (95% confidence interval: 97.64-99.99%);
– Overall diagnostic sensitivity: 99.53% (95% confidence interval: 99.08-99.80%).

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. Rubella and pregnancy: diagnosis, management and outcomes. Bouthry E, Picone O, Hamdi G, Grangeot-Keros L, Ayoubi JM, Vauloup-Fellous C. Prenat Diagn. 2014 Dec; 34(13):1246-53. doi: 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://apps.who.int/iris/bitstream/handle/10665/272807/9789241210201eng.pdf?ua=1&ua=1 (accessed Aug 9, 2019).
5. WHO. Global and regional immunization profile.2018.http://www.who.int/immunization/monitoring_surveillance/data/ gs_gloprofile.pdf?ua=1 (accessed Jan 19, 2018).
6. Bowen et al., Clinical Biochemistry, 43, 4-25, 2010

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