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Changes: §4, §6;
Deletions: -

LIAISON® Measles IgG (REF 318810)

1. INTENDED USE

The LIAISON® Measles IgG assay uses chemiluminescence immunoassay (CLIA) technology for the semi-quantitative determination of specific IgG antibodies to measles virus in human serum or plasma samples. It is intended to be used as an aid in the determination of serological status to measles virus.
The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Measles is an acute viral illness caused by a morbillivirus of the paramyxovirus family and is one of the most easily transmitted diseases. Transmission is primarily by large droplet spread or direct contact with nasal or throat secretions from an infected person (1).

After infection, measles virus invades the respiratory epithelium of the nasopharynx and spreads to the regional lymph nodes. After two to three days of replication in these sites, primary viraemia widens the infection to the reticulo-endothelial system. Following further replication, secondary viraemia occurs five to seven days after infection and lasts four to seven days. During this viraemia, infection and further virus replication may occur in skin, conjunctivae, respiratory tract and other organs, including spleen, thymus, lung, liver, and kidney. Viraemia peaks 11-14 days after infection, and then declines rapidly over a few days (2).

Prior to vaccine availability, measles was mostly a disease of childhood, but measles vaccination programs (part of measles, mumps, rubella [MMR] vaccination) have had a marked effect on the incidence of the disease and the complications associated with it. After prolonged periods of high vaccine coverage in developed countries, measles transmission now occurs mainly in people that have never been vaccinated and in older children who did not seroconvert following vaccination. Measles outbreaks can still occur in countries with high immunization coverage. Such outbreaks demonstrate an immunity gap in the population involved (1, 3).

Clinically, the diagnosis of measles is supported if Koplik's spots are detected and if the rash progresses from the head to the trunk and out to the extremities (6). The non-specific nature of the prodromal signs and the existence of mild cases, however, make clinical signs unreliable as the sole diagnostic criteria of measles disease. As disease prevalence falls, many medical practitioners are inexperienced in recognizing measles, increasing the need for laboratory serological method of distinguishing measles from other clinically similar diseases (4).

Both IgM and IgG antibodies are synthesized during the primary immune response and can be detected in the serum within a few days of rash onset. IgM antibody levels peak after about seven to ten days and then decline rapidly, being rarely detectable after six to eight weeks. IgM is generally not detected in an immune individual following re-exposure to measles virus (5). Re-exposure to the measles virus induces a strong anamnestic immune response with a rapid boosting of IgG antibodies, which prevents clinical disease (6).

3. PRINCIPLE OF THE PROCEDURE

The method for semi-quantitative determination of specific IgG to measles virus is an indirect *sandwich* chemiluminescence immunoassay (CLIA). Recombinant measles virus antigen is used for coating magnetic particles (solid phase) and mouse monoclonal antibody directed against human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, measles 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 measles 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 indicates the presence or absence of IgG to measles virus in calibrators, samples or controls.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles coated with recombinant measles virus nucleoprotein (obtained in baculovirus), BSA, PBS buffer < 0.1% sodium azide.
Calibrator 1 (0.55 mL)	CAL 1	Human serum/defibrinated plasma containing low IgG levels to measles virus, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Calibrator 2 (0.55 mL)	CAL 2	Human serum/defibrinated plasma containing high IgG levels to measles virus, BSA, phosphate buffer, EDTA, detergents, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Specimen diluent (2 x 27 mL)	DIL SPE	Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, an inert blue dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative, BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives, an inert yellow dye.
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® 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® Measles IgG controls (negative and positive) (REF 318811).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

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 labelled as follows:

REAGENTS:	CAL 1, CAL 2, 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:

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 weeks.
  After this period, it is still possible to keep on using the reagent integral provided that the controls are found within the expected ranges.
- 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

Either human serum or plasma may be used. The anticoagulants potassium EDTA and sodium heparin have been tested and may be used with this assay. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity, lipaemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. If the assay is performed within nine days of sample collection, the samples may be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (–20°C or below). If samples are stored frozen, mix thawed samples well before testing. Eight samples with different reactivity were stored for nine days at 2-8°C and seven samples underwent six freeze-thaw cycles. The results showed no significant differences. The minimum volume required for a single determination is 170 µL 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.
– 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 local 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 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 coated magnetic particles and specimen diluent 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® Measles IgG 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 beyond eight 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 measles virus IgG concentrations expressed as arbitrary units (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. 5 to 300 AU/mL measles virus IgG.

The cut-off value discriminating between the presence and the absence of measles virus IgG is 15.0 AU/mL. Sample results should be interpreted as follows:

Samples with measles virus IgG concentrations below 13.5 AU/mL should be graded *negative*.

Samples with measles virus IgG concentrations ranging between 13.5 and 16.5 AU/mL 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 measles virus IgG concentrations equal to or above 16.5 AU/mL should be graded *positive*.

A negative result for IgG antibodies to measles virus generally indicates that the individual has not been infected and is susceptible to measles. However it does not exclude the possibility of acute measles, because the infection may be in its very early stage and the patient may be still unable to synthesize measles virus specific antibodies, or the antibodies may be present in undetectable levels. It should be underlined that the test scores negative during the first weeks after infection. If clinical exposure to measles virus is suspected despite a negative or equivocal finding, but the subject has no history of measles, nor has been previously vaccinated a second sample should be collected and tested no less than one to two weeks later.

A positive result for IgG antibodies to measles virus generally indicates past exposure to measles virus or previous vaccination thereby inferring immunity. A single specimen, however, can only help estimate the serological status of the individual.

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® measles test is used in conjunction with other manufacturers' assays for detection of specific measles serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Bacterial contamination or heat inactivation of the specimens may affect the test results.

Test results are reported semi-quantitatively as positive or negative for the presence of measles 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 and other diagnostic procedures as well as in association with medical judgement.

Specimens from patients receiving preparations of mouse monoclonal antibodies for therapy or diagnosis may contain human anti-mouse antibodies (HAMA). Such specimens may interfere in a monoclonal antibody-based immunoassay and their results should be evaluated with care.

Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON®, LIAISON® XL or LIAISON® XS).

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.

Reference to WHO standard. The cut-off value of LIAISON® Measles IgG immunoassay equates to 175 mIU/mL WHO Third International Standard for Anti-Measles, NIBSC code: 97/648. This result should provide further information and must be considered merely indicative.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (potassium EDTA, sodium heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), albuminaemia (up to 5.0 g/dL albumin), or by up to six freeze-thaw cycles of samples. The use of citrate plasmas causes a decrease of the signal with consequent underestimation of results (of the order of 20%).

Cross-reactions. The cross-reactivity study for the LIAISON® Measles IgG assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to those of measles (hCMV, EBV, rubella virus, parvovirus B19, mumps virus, *Toxoplasma gondii*), from antibodies to other organisms that may cause infectious diseases (HSV, VZV, HAV) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies). Samples for these studies were pre-screened with another commercially available measles virus IgG assay. Samples that were seronegative for measles virus IgG 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.

Clinical condition	Number of expected negative samples	LIAISON® positive or equivocal results
hCMV IgG antibodies	9	0
VZV IgG antibodies	10	0
Parvovirus B19 IgG antibodies	14	0
HSV-1/2 IgG antibodies	7	0
Rubella virus IgG antibodies	7	0
EBV IgG antibodies	15	0
Mumps virus IgG antibodies	10	0
HAV antibodies	4	0
Toxoplasma gondii IgG antibodies	6	0
Anti-nuclear autoantibodies (ANA)	5	0
Total	87	0

None of the 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 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	C	D	E	Positive control
Number of determinations	20	20	20	20	20	20
Mean (AU/mL)	4.1	6.5	10.4	32.1	66.8	83.2
Standard deviation (AU/mL)	0.2	0.4	0.4	2.1	5.0	4.6
Coefficient of variation (%)	5.5	5.8	4.3	6.4	7.5	5.5
Min. value (AU/mL)	3.8	6.1	9.1	27.9	57.9	77.0
Max. value (AU/mL)	4.6	7.4	11.2	35.6	73.2	91.3

Reproducibility. Eighty replicates were performed in different days (two runs in duplicate per day for twenty days) 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	F	G	H	I	J	K	Positive control
LOT No. 01							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	7.2	31.2	67.8	85.2	145.4	195.5	85.7
Standard deviation (AU/mL)	0.7	3.0	7.4	7.9	17.2	21.4	6.9
Coefficient of variation (%)	9.3	9.6	10.9	9.3	11.9	10.9	8.0
Min. value (AU/mL)	5.9	20.5	38.2	60.1	60.1	138.0	69.4
Max. value (AU/mL)	8.7	37.2	82.4	101.0	175.0	241.0	98.5
LOT No. 02							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	5.8	34.5	83.4	104.9	223.0	286.2	114.0
Standard deviation (AU/mL)	0.5	3.5	8.2	10.6	21.7	21.5	11.2
Coefficient of variation (%)	8.9	10.2	9.9	10.1	9.7	7.5	9.8
Min. value (AU/mL)	4.8	22.6	58.6	68.3	140.0	206.0	92.5
Max. value (AU/mL)	7.1	42.1	101.0	135.0	279.0	300.0	143.0
LOT No. 03							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	6.8	32.0	73.8	92.5	168.6	213.6	93.2
Standard deviation (AU/mL)	0.7	3.6	7.8	8.9	17.1	26.1	7.7
Coefficient of variation (%)	9.9	11.1	10.5	9.6	10.1	12.2	8.3
Min. value (AU/mL)	5.6	19.4	50.8	66.4	114.0	130.0	77.4
Max. value (AU/mL)	8.7	40.9	93.3	110.0	207.0	264.0	111.0
Inter-lot coefficient of variation (%)	9.4	10.3	10.4	9.7	10.6	10.2	8.7

Reproducibility - Site 2	F	G	H	I	J	K	Positive control
LOT No. 01							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	6.0	28.5	62.2	79.1	137.3	177.7	76.2
Standard deviation (AU/mL)	0.5	2.7	4.3	7.3	13.3	18.7	6.4
Coefficient of variation (%)	7.7	9.6	7.0	9.3	9.7	10.5	8.4
Min. value (AU/mL)	5.2	20.6	50.3	52.8	103.0	111.0	61.8
Max. value (AU/mL)	8.0	35.8	72.4	94.5	165.0	207.0	93.7
LOT No. 02							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	6.0	28.2	67.8	80.3	152.0	197.7	85.0
Standard deviation (AU/mL)	0.6	3.9	7.9	9.8	14.5	15.4	7.5
Coefficient of variation (%)	9.7	13.9	11.6	12.2	9.6	7.8	8.8
Min. value (AU/mL)	4.4	18.2	50.8	57.7	104.0	164.0	66.1
Max. value (AU/mL)	8.3	41.4	97.3	116.0	188.0	244.0	115.0
LOT No. 03							
Number of determinations	80	80	80	80	80	80	80
Mean (AU/mL)	5.2	29.2	74.9	95.6	190.7	265.2	96.4
Standard deviation (AU/mL)	0.4	3.3	7.9	10.6	20.9	30.5	7.4
Coefficient of variation (%)	7.0	11.2	10.6	11.1	11.0	11.5	7.6
Min. value (AU/mL)	4.3	20.4	52.5	64.1	131.0	153.0	76.2
Max. value (AU/mL)	6.0	37.4	94.8	122.0	241.0	300.0	114.0
Inter-lot coefficient of variation (%)	8.1	11.5	9.7	10.9	10.1	9.9	8.3

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	3	Positive control
Number of determinations	20	20	20	20
Mean (AU/mL)	6.8	33.9	74.1	98.2
Standard deviation	0.55	2.1	3.1	4.5
Coefficient of variation (%)	8.0	4.3	4.2	4.6
Min. value (AU/mL)	5.8	28.9	67.0	92.1
Max. value (AU/mL)	7.7	39.1	80.4	107.0

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

Reproducibility	4	5	6	Positive control
Number of determinations	20	20	20	20
Mean (AU/mL)	6.1	41.6	88.1	118.4
Standard deviation	0.86	5.5	8.9	7.4
Coefficient of variation (%)	14.1	13.3	10.1	6.3
Min. value (AU/mL)	5.0	32.1	71.6	103.0
Max. value (AU/mL)	7.8	52.5	102.0	132.0

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® Measles IgG Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.
A coded panel comprised of 10 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 Measles IgG 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.

Repeatability	7	8	9	10	11	12	13	14	15	16	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90	90	90
Mean (AU/mL)	11.3	6.99	25.4	25.5	97.6	65.1	71.0	125	121	168	86.8
Standard deviation	0.266	0.195	0.529	0.480	1.885	1.515	1.805	2.716	2.680	4.583	2.164
Coefficient of variation (%)	2.3	2.8	2.1	1.9	1.9	2.3	2.5	2.2	2.2	2.7	2.5
Min. value (AU/mL)	9.94	5.74	21.4	22.3	83.7	57.5	62.6	109	109	144	74.6
Max. value (AU/mL)	12.9	8.09	27.7	27.6	107	77.5	78.5	140	136	197	99.6

Reproducibility.

Reproducibility	7	8	9	10	11	12	13	14	15	16	Positive control
Number of determinations	90	90	90	90	90	90	90	90	90	90	90
Mean (AU/mL)	11.3	6.99	25.4	25.5	97.6	65.1	71.0	125	121	168	86.8
Standard deviation	0.585	0.360	1.143	1.007	3.777	2.358	2.939	4.481	3.843	5.869	4.359
Coefficient of variation (%)	5.1	5.2	4.5	3.9	3.9	3.6	4.1	3.6	3.2	3.5	5.0
Min. value (AU/mL)	9.94	5.74	21.4	22.3	83.7	57.5	62.6	109	109	144	74.6
Max. value (AU/mL)	12.9	8.09	27.7	27.6	107	77.5	78.5	140	136	197	99.6

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).
Analysis of saturation effect was evaluated by testing three high-titred samples positive for measles virus IgG. 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 529 unselected specimens collected from a European laboratory routine. Different groups of subjects were included (300 unselected individuals, 50 children ages 0-8 years, 117 subjects with serology suggestive of susceptibility to infection, 62 patients with serology suggestive of acute infection). The specimens were tested by a comparison method and consensus with additional clinical and serological data was applied to define the expected results. Twelve specimens were unresolved by the reference method and therefore were not included in the data analysis.

Three positive, two equivocal and 189 negative results were observed in the expected negative population studied - diagnostic specificity: 97.4% (189/194) (95% confidence interval: 94.1-99.2%).

Thirteen negative, four equivocal and 306 positive results were observed in the expected positive population studied - diagnostic sensitivity: 94.7% (306/323) (95% confidence interval: 91.7-96.9%).

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