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Deletions: §14;

LIAISON® Chlamydia trachomatis IgG (REF 310570)

1. INTENDED PURPOSE

The LIAISON® Chlamydia trachomatis IgG assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro semi-quantitative determination of specific IgG antibodies to *Chlamydia trachomatis* in human serum or plasma samples. The assay is intended as an aid to the diagnosis of current, chronic and past Chlamydia trachomatis infections in suspected patients, including children. The test has to be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Chlamydia trachomatis is a gram-negative and obligatory intracellular bacterium causing the most commonly reported sexually transmitted infection (STI) in Europe and United States, with high incidence rates in young people between 15 and 24 years of age[1, 2].

Within the C. trachomatis species, there are three human biological variants: agents of trachoma[9, 12], agents of lymphogranuloma venereum (LGV)[7, 11] and agents of sexually transmitted genital infections as cervicitis, endometritis, salpingitis and urethritis. According to the World Health Organization (WHO), it is estimated that over 100 million new cases of genital C. trachomatis infections (chlamydia) occur per year[3], with more than two thirds of cases in developing countries[2], and approximately 40% in young women[6]. Transmission occurs through direct mucosal contact or during birth; while eye infection is caused by direct contact with eye secretions or nasal discharge[4, 5]. C. trachomatis infection is mostly asymptomatic, with only 30% of the infected population developing symptoms of genital chlamydia after an incubation period of up to two weeks. 80% of infected women develops important sequelae in the uterine cervix, urethra, rectum and also in the lungs and eyes if not accurately identified and treated[3].

Several diagnostic methods are available, such as cell culture isolation, antigen detection methods and nucleic acid amplification tests (NAATs) for direct detection of C. trachomatis. The availability of a serological assay for IgG or IgA is useful in different situations, such as to determine if a patient had a previous infection[8, 10, 13].

Chlamydia IgG immunoglobulin antibodies persist for years and are used as markers of a previous C. trachomatis infection. C. trachomatis IgG antibodies are usually detected after 2–3 weeks from the primary infection and are considered an important marker of past infection[14]. In infertile women, their detection is relevant to assess a previous C. trachomatis infection as the possible cause of infertility[15].

3. PRINCIPLE OF THE PROCEDURE

The method for semi-quantitative determination of specific IgG to *Chlamydia trachomatis* is an indirect chemiluminescence immunoassay (CLIA). Assay Buffer 1 contains biotinylated *Chlamydia trachomatis* specific synthetic peptides, while magnetic particles (solid phase) are coated with streptavidin, and a mouse monoclonal antibody directed against human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, *Chlamydia trachomatis* antibody, if present in calibrators, samples or controls, binds to biotinylated peptides and then on to the solid phase by biotin-streptavidin link. During the second incubation, the mouse monoclonal antibody reacts with any human *Chlamydia Trachomatis* 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 *Chlamydia trachomatis* in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles (≥ 0.25% solid) coated with streptavidin, BSA, PBS buffer, < 0.1% sodium azide.
Calibrator 1 (0.55 mL)	CAL 1	Human serum/plasma containing low *Chlamydia trachomatis* IgG levels (approx 22.5 AU/mL), Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, 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/plasma containing medium *Chlamydia trachomatis* IgG levels (approx. 75 AU/mL), Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, an inert blue dye. The calibrator concentrations (AU/mL) are referenced to an in-house antibody preparation.
Assay buffer 1 (23.5 mL)	BUF 1	*Chlamydia trachomatis* specific synthetic peptides (approx 76 ng/mL), Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, an inert blue dye.
Conjugate (23.5 mL)	CONJ	Mouse monoclonal IgG to human IgG (> 10 ng/mL) conjugated to an isoluminol derivative, foetal calf serum, phosphate buffer, 0.2% ProClin™ 300, preservatives, an inert red 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.

*(LIAISON® and LIAISON® XL)

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).

Additionally required materials

LIAISON® Chlamydia trachomatis IgG controls (negative and positive) (REF 310571).

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 follow:

REAGENTS:	CONJ
CLASSIFICATION:	Skin sens. 1A H317 STOT RE 2 H373 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark  GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H373 May cause damage to organs (kidney) through prolonged or repeated exposure. 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. P314 Get medical advice/attention if you feel unwell.
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); Ethylene glycol.

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. Incomplete magnetic particle resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

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

Loading of integral into the reagent area

LIAISON® Analyzer

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

LIAISON® XL Analyzer

- LIAISON® XL Analyzer is 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**: Stability eight (8) weeks.
- Use always the same analyzer for a reagent integral already opened.
- Use the storage rack provided with the analyzer for upright storage of the reagent integral.
- Do not freeze.
- Keep upright for storage to facilitate later proper resuspension of magnetic particles.
- Keep away from direct light.

9. SPECIMEN COLLECTION AND PREPARATION

The correct specimen type must be used in the assay. Following matrices have been tested and may be used:
- serum,
- lithium heparin plasma,
- K2-EDTA,
- sodium citrate.

Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation, carefully following the tube manufacturers' instructions and according to good laboratory practices.

Centrifugation conditions of collection tubes may vary depending on the manufacturer. A minimum of 1,000 g for 10 minutes is reported. Use of centrifugation conditions should be evaluated and validated by the laboratory.

Package and label specimens in compliance with applicable regulations covering the transport of clinical specimens and infectious substances. Specimens may be shipped on dry ice (frozen), on wet ice (for 2°-8°C), following the sample storage limitations described below. Uncontrolled transport conditions (in terms of temperature and time) may cause inaccurate analytical results. During validation studies, specimen collection tubes commercially available at the time of testing were used. Therefore, not all collection tubes from all manufacturers have been evaluated. Blood collection devices from various manufacturers may contain substances which could affect the test results in some cases (Bowen et al., Clinical Biochemistry, 43, 4-25, 2010).

A dedicated study on storage limitations was performed on serum or plasma specimens removed from clot, red cells or gel separator. The following storage conditions showed no significant differences:
- 2°-8°C for 7 days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
- Up to five (5) freeze-thaw cycles, however multiple freeze thaw cycles should be avoided;
- The room temperature storage 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

Assay specific calibrator testing 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 eight (8) weeks before.
- The analyzer has been serviced.
- Control values lie outside the expected ranges.
- LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.
  LIAISON® XL Analyzer: Calibrator values are stored in the Radio Frequency IDentification transponder (RFID Tag) of the reagent integral.

11. ASSAY PROCEDURE

Strict adherence to the relevant 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 Analyzer. Each test parameter is identified via information encoded in the reagent integral Radio Frequency IDentification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

The analyzer operations are as follows:
1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense assay buffer 1.
3. Dispense coated magnetic particles.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Kit and measure the light emitted.

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® Chlamydia trachomatis IgG controls (REF 310571)
(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) 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, the patient's 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 the quality control materials used.

13. INTERPRETATION OF RESULTS

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

The analyzer automatically calculates *Chlamydia trachomatis* IgG concentrations expressed as arbitrary units (AU/mL) and grades the results. For details, refer to the relevant analyzer operator's manual.

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

Assay range. 5 to 250 AU/mL *Chlamydia trachomatis* IgG.

The cut-off value discriminating between the presence and the absence of *Chlamydia trachomatis* IgG is 10 AU/mL.

Sample results should be interpreted as follows:
Samples with *Chlamydia trachomatis* IgG concentrations below 9 AU/mL should be graded *negative*.

Samples with *Chlamydia trachomatis* IgG concentrations ranging between 9 and 11 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 *Chlamydia trachomatis* IgG concentrations equal to or above 11 AU/mL should be graded *positive*.

A negative result for IgG antibodies to *Chlamydia trachomatis* generally indicates that the individual has not been infected and is susceptible to *Chlamydia trachomatis*. However, it does not exclude the possibility of acute *Chlamydia trachomatis*, because the infection may be in its very early stage and the patient may be still unable to synthesize anti-*Chlamydia trachomatis* specific antibodies, or the antibodies may be present in undetectable levels.

A positive result for IgG antibodies to *Chlamydia trachomatis* can either indicate current, chronic or past infections.

13.1. Interpretation of IgG and IgA combined results obtained in single specimens

IgG results	IgA results	Interpretation of results
Negative	Negative	No evidence of infection. A negative result for antibodies to *Chlamydia trachomatis* generally indicates that the patient has not been infected, but does not exclude the possibility of infection.
Positive	Negative or equivocal	May indicate past or current infection.
Equivocal	Equivocal	Test a second sample, collected no less than one or two weeks later, in parallel with the first sample.
Positive	Positive	May indicate acute or chronic infection.
Negative	Positive	May indicate acute or chronic infection.

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® Chlamydia trachomatis test is used in conjunction with other manufacturers' assays for detection of specific *Chlamydia trachomatis* 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 equivocal or negative for the presence of *Chlamydia trachomatis* 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.

Specimens from patients receiving therapeutic doses of Biotin (Vitamin H, B7 or B8) may interfere in immunoassays based on biotinylated reagents. No interference was observed testing Biotin serum concentration up to 10 ng/mL with LIAISON Chlamydia trachomatis IgG assay (for details, refer to §15.1).

Integrals may not be exchanged between analyzer types (LIAISON® and LIAISON® XL). 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 a specific analyte in the presence of potentially interfering factors in the sample matrix (e.g. anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (potassium EDTA, lithium heparin, sodium citrate), haemolysis (up to 10 mg/mL haemoglobin), lipaemia (up to 30 mg/mL triglycerides), bilirubinaemia (up to 0.2 mg/mL bilirubin), serum levels of Vitamin H up to 10 ng/mL or by up to five freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® Chlamydia trachomatis IgG assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to *Chlamydia trachomatis* infection (*Chlamydia pneumoniae, Mycoplasma pneumoniae, Treponema pallidum*), from other conditions that may result from atypical immune system activity (rheumatoid factor RF, anti-nuclear autoantibodies ANA, human anti-mouse antibodies HAMA), from antibodies to other organisms that may cause infectious diseases (*Toxoplasma gondii*, hCMV, rubella virus, parvovirus B19, EBV, measles virus, mumps virus, VZV, HCV, HBV, HIV). Samples for these studies were pre-screened with another commercially available *Chlamydia trachomatis* IgG assay. Samples that were seronegative for *Chlamydia trachomatis* 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	8	0
VZV antibodies	5	0
Parvovirus B19 IgG antibodies	11	0
Measles virus IgG antibodies	6	0
Rubella virus IgG antibodies	11	0
EBV antibodies	6	0
Mumps virus IgG antibodies	6	0
Toxoplasma gondii IgG antibodies	6	0
HCV antibodies	5	0
HBV antibodies	6	0
HIV antibodies	5	0
Rheumatoid factor (anti-Fc immunoglobulin)	5	0
Anti-nuclear antibodies (ANA)	6	0
Human anti-mouse antibodies (HAMA)	9	0
Chlamydia pneumoniae antibodies	57	3
Mycoplasma pneumoniae IgG antibodies	6	0
Treponema pallidum antibodies	19	0
Total	177	3

There was no conclusive evidence of cross-reactivity observed; however, the possibility of cross-reactivity with *Chlamydia pneumoniae* antibodies 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	Positive control
Number of determinations	20	20	20
Mean (AU/mL)	20.5	47.1	17.3
Standard deviation (AU/mL)	1.4	1.8	1.1
Coefficient of variation (%)	6.9	3.8	6.5
Min. value (AU/mL)	17.2	42.8	14.9
Max. value (AU/mL)	22.5	51.1	19.3

Reproducibility. Twenty replicates were performed in different days (two runs in singlicate per day for ten 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	A	B	Positive control
LOT No. 01			
Number of determinations	20	20	20
Mean (AU/mL)	23.1	52.1	20.7
Standard deviation (AU/mL)	2.3	4.9	1.5
Coefficient of variation (%)	9.7	9.5	7.2
Min. value (AU/mL)	19.8	42.7	17.3
Max. value (AU/mL)	28.3	59.2	23.6
LOT No. 02			
Number of determinations	20	20	20
Mean (AU/mL)	22.3	53.3	19.8
Standard deviation (AU/mL)	2.0	3.8	1.5
Coefficient of variation (%)	9.1	7.0	7.8
Min. value (AU/mL)	19.8	42.7	16.4
Max. value (AU/mL)	28.3	59.9	22.2
LOT No. 03			
Number of determinations	20	20	20
Mean (AU/mL)	24.8	49.7	24.7
Standard deviation (AU/mL)	3.3	4.1	1.3
Coefficient of variation (%)	13.4	8.2	5.3
Min. value (AU/mL)	19.8	42.7	22.0
Max. value (AU/mL)	24.5	59.9	27.3
Interlot Coefficient of variation (%)	5.5	4.1	12.1

Reproducibility - Site 2	A	B	Positive control
LOT No. 01			
Number of determinations	20	20	20
Mean (AU/mL)	21.2	51.5	22.2
Standard deviation (AU/mL)	2.1	3.8	2.12
Coefficient of variation (%)	9.9	7.4	9.6
Min. value (AU/mL)	18.8	41.6	23.8
Max. value (AU/mL)	26.2	57.4	29.7
LOT No. 02			
Number of determinations	20	20	20
Mean (AU/mL)	20.3	55.3	21.2
Standard deviation (AU/mL)	2.2	4.3	2.3
Coefficient of variation (%)	10.9	7.8	9.5
Min. value (AU/mL)	17.4	44.9	22.3
Max. value (AU/mL)	25.0	64.8	30.5
LOT No. 03			
Number of determinations	20	20	20
Mean (AU/mL)	24.4	49.8	28.3
Standard deviation (AU/mL)	2.2	4.4	2.7
Coefficient of variation (%)	8.8	8.8	9.6
Min. value (AU/mL)	21.4	35.7	23.5
Max. value (AU/mL)	27.7	55.6	31.7
Interlot Coefficient of variation (%)	9.8	5.4	16.1

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of a specific analyte, were assayed to estimate repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

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

Repeatability	1	2	Positive control
Number of determinations	20	20	20
Mean (AU/mL)	19.7	47.2	22.5
Standard deviation	1.2	2.2	0.8
Coefficient of variation (%)	6.1	4.7	3.3
Min. value (AU/mL)	17.4	43.0	21.1
Max. value (AU/mL)	22.5	53.0	23.9

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

Reproducibility	1	2	Positive control
LOT No. 01			
Number of determinations	20	20	20
Mean (AU/mL)	29.7	66.4	25.5
Standard deviation (AU/mL)	1.8	5.2	2.0
Coefficient of variation (%)	6.1	7.8	7.9
Min. value (AU/mL)	26.7	60.0	21.4
Max. value (AU/mL)	33.0	81.6	28.5
LOT No. 02			
Number of determinations	20	20	20
Mean (AU/mL)	28.5	65.7	25.2
Standard deviation (AU/mL)	2.0	6.9	1.4
Coefficient of variation (%)	6.9	10.6	5.5
Min. value (AU/mL)	25.5	56.5	22.9
Max. value (AU/mL)	32.7	78.6	28.4
LOT No. 03			
Number of determinations	20	20	20
Mean (AU/mL)	27.6	54.7	26.2
Standard deviation (AU/mL)	2.1	7.4	2.9
Coefficient of variation (%)	7.6	13.5	11.0
Min. value (AU/mL)	23.9	44.1	21.0
Max. value (AU/mL)	31.4	69.9	32.0
Interlot Coefficient of variation (%)	3.7	10.5	2.0

15.4. Trueness by recovery test

5 serum samples were prepared from an high and a low (close to low assay range limit) IgG Chlamydia t. samples mixed in different proportions (low sample neat, 1:2, 1:1, 2:1 and high sample neat) and assayed. Percent recoveries were determined from results of undiluted samples. Percentage of recovery lies in the range 83.6% and 96.8%. Measured versus expected IgG Chlamydia t. concentrations were analyzed by linear regression; a slope = 1.0114 and R = 0.997 (R2 = 0.9939) were obtained.

Sample	Low (%)	High (%)	Expected (AU/mL)	Measured (AU/mL)	Recovery (%)
CHTR -S1	100	-	6.12	6.12	-
CHTR -S2	66.7	33.3	67.4	56.4	83.6
CHTR -S3	50	50	99.1	88.0	88.8
CHTR -S4	33.3	66.7	130.1	126.0	96.8
CHTR -S5	-	100	192.0	192.0	-
				Average Recovery (%)	89.8

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 the saturation effect was evaluated by testing three high-titred samples positive for *Chlamydia trachomatis* IgG. All samples resulted in high concentration values as expected, indicating no sample misclassification.

15.6. Analytical and functional sensitivity

The Limit of Blank (LoB) for the LIAISON® Chlamydia trachomatis IgG assay is 2.78 AU/mL. Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.654 standard deviations. Following the method from CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Chlamydia trachomatis IgG assay is 3.28 AU/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® Chlamydia trachomatis IgG assay is 3.28 AU/mL.

15.7. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 622 unselected specimens collected from the routine testing of two European laboratories. Among them, 49 specimens from children (age 0-12 years) were included. The specimens were tested by a reference CE mark method, and consensus with additional clinical and serological data was applied to define the expected results.

Ten specimens were unresolved even after resolution and therefore not included in the data analysis.

Three positive, one equivocal and 225 negative results were observed in the expected negative population studied - diagnostic specificity: 98.2% (225/229) (95% confidence interval: 95.6-99.5%).

Fourteen negative, four equivocal and 365 positive results were observed in the expected positive population studied - diagnostic sensitivity: 95.3% (365/383) (95% confidence interval: 92.7-97.2%).

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.

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