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Changes: -
Deletions: -

LIAISON® Bordetella pertussis Toxin IgG (REF 318850)

1. INTENDED USE

The LIAISON® Bordetella pertussis Toxin IgG kit uses chemiluminescent immunoassay (CLIA) technology for the quantitative determination of IgG antibodies to Bordetella pertussis Toxin in human serum and plasma samples.
The test has to be performed on the LIAISON® Analyzer family.

2. SUMMARY AND EXPLANATION OF THE TEST

Pertussis, a cough illness commonly known as whooping cough, is a bacterial respiratory infection caused by *Bordetella pertussis* Gram-negative bacillus, an exclusively human pathogen which can affect people of all ages. The illness is characterized by a prolonged cough often accompanied by an inspiratory whoop. The genus Bordetella (B.) encompasses four known species: *B. pertussis*, which causes whooping cough, B. parapertussis, which causes a whooping cough syndrome, B. bronchiseptica and B. avium. Of these human and animal pathogens, *B. pertussis* and more rarely B. parapertussis are medically relevant. They are distributed worldwide and are transmitted from person to person by droplet infection. *Bordetella pertussis* overcomes the local immune defence mechanisms of the human upper respiratory tract. The bacteria bind via various adhesions to the cells of the ciliated epithelium without reaching the epithelium or bloodstream. Besides the capsule, which protects the pathogen from inactivation by complement, there are functionally two groups of virulence factors: adhesins and toxins, of which the two most important are filament haemagglutin (FHA) and pertussis toxin (PT). The PT is the exotoxin responsible for many physiological, immunological and pharmacological effects. In contrast to other exotoxins of the species *Bordetella*, that show high cross-reactivities in serum diagnostics, the Pertussis Toxin is highly specific.
After an incubation time of around 7 to 14 days, whooping cough begins with an uncharacteristic catarrhal stage, which lasts for about 1 to 2 weeks. Then the convulsive stage develops, lasting 2 to 3 weeks with typical paroxysmal, coughing attacks, frequently followed by stridor with possible vomiting. The coughing attacks frequently occur during the night. During both of these stages pathogen is coughed out. Transmission via contaminated objects cannot be excluded. The decreasing stage follows, which lasts for several weeks, with continual diminishment of coughing attacks. Complications such as secondary pneumonia and otitis media are possible, especially in children. Season and climate have no influence on the frequency of the disease. The infection leaves behind a specific immunity, which declines, however, after several decades. The disease is known in adults and adolescents, but is under-diagnosed, thus leading to reservoirs for infection of unvaccinated infants or susceptible subjects. Serological diagnosis of pertussis should only be attempted in patients with symptoms compatible with pertussis. In infants, older vaccinated children, adolescents and adults the clinical course may not be typical, and prolonged coughing may be the only symptom. In these cases, diagnosis of pertussis requires laboratory methods for confirmation.

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgG to *Bordetella pertussis* Toxin is an indirect chemiluminescence immunoassay (CLIA). Magnetic particles (solid phase) are coated with *B. pertussis* Toxin and mouse monoclonal antibody directed against human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, *Bordetella pertussis* Toxin antibody, if present in calibrators, samples or controls, binds to the solid phase. During the second incubation, the mouse monoclonal antibody reacts with any human *Bordetella pertussis* Toxin 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 *Bordetella pertussis* Toxin IgG in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.5 mL)	SORB	Magnetic particles coated with purified Pertussis Toxin, BSA, Phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (0.55 mL)	CAL 1	Human serum/plasma containing low *Bordetella pertussis* Toxin IgG levels, 0.2% ProClin™ 300, preservatives. The calibrator concentrations (IU/mL) are referenced to first International WHO standard (WHO International Standard Pertussis Antiserum, human, 1st IS NIBSC Code 06/140) preparation.
Calibrator 2 (0.55 mL)	CAL 2	Human serum/plasma containing medium *Bordetella pertussis* Toxin IgG levels, 0.2% ProClin™ 300, preservatives, an inert blue dye. The calibrator concentrations (IU/mL) are referenced to first International WHO standard (WHO International Standard Pertussis Antiserum, human, 1st IS NIBSC Code 06/140) preparation.
Specimen diluent (27 mL)	DIL SPE	Casein, BSA, phosphate buffer, EDTA, detergents, preservatives, an inert blue dye.
Conjugate (23.5 mL)	CONJ	Mouse monoclonal IgG to human IgG 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.

Materials required but not provided (system related)

LIAISON® XL Analyzer	LIAISON® Analyzer
LIAISON® XL Cuvettes (REF X0016). LIAISON® XL Disposable Tips (REF X0015). LIAISON® XL Starter Kit (REF 319200). – 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). 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® Bordetella pertussis Toxin IgG controls (Non-Reactive and Reactive) (REF 318851).

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 labeled as follows:

REAGENTS:	CAL 1, CAL 2	CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412	Skin sens. 1A H317 STOT RE 2 H373 Aquatic chronic 3 H412
SIGNAL WORD:	Warning	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 – Exclamation mark	  GHS07 Exclamation mark GHS08 Health hazard
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.	H317 May cause an allergic skin reaction. 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.	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).	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.

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 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

Either human serum or plasma may be used. The anticoagulants sodium citrate, potassium EDTA, lithium heparin, have been tested and may be used with this assay. The correct specimen type must be used in the assay.

Follow tube manufacturers' instructions carefully when using collection containers. Blood should be collected aseptically by venipuncture and the serum or plasma separated from clot, red cells or gel separator, after centrifugation.

Centrifugation conditions range from 1,000 to 3,000 g for 10 minutes. Conditions may vary depending on tube manufacturers' recommendations. Use of alternate centrifugation conditions should be evaluated and validated by the laboratory.

Before shipping specimens, serum or plasma specimens should be removed from clot, red cells or gel separator. Specimens may be shipped in dry ice (frozen), in wet ice (for 2°-8°C) or at room temperature (20°-25°C), by following sample storage limitations described below. **Uncontrolled transport conditions (in terms of temperature and time) can 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).

Concerning storage limitations, if the assay is performed within seven days of sample collection, the samples removed from red cells, clot or gel separator may be kept at 2°-8°C; otherwise they should be aliquoted and stored deep-frozen (–20°C or below). Ten samples with different reactivity were stored for seven days at 2°-8°C and ten samples underwent five freeze-thaw cycles. The results showed no significant differences; however multiple freeze-thaw cycles should be avoided. If samples are stored frozen, mix thawed samples well before testing. Ten serum specimens were also stored at room temperature (20°-25°C) up to 48h and the results showed no significant differences. However, room temperature storage conditions should be evaluated and validated by the laboratory.

Samples removed from red cells, clot or gel separator having particulate matter, fibrin, turbidity, lipaemia, or erythrocyte debris, specimens that have been stored at room temperature (20°-25°C), or frozen and thawed, or samples requiring repeat testing, require clarification by further centrifugation (it's recommended 10,000 g for 10') before testing, to improve consistency of results. Specimens with a lipid layer on the top should be transferred in 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. Check for and remove air bubbles before assaying.

The minimum volume required for determination is 170 µL 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 four 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).

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 the following:

1. It dispenses calibrators, controls or specimens into the reaction module.
2. It dispenses specimen diluent.
3. It dispenses coated magnetic particles.
4. It incubates.
5. It washes with Wash/System liquid.
6. It dispenses conjugate into the reaction module.
7. It incubates.
8. It washes with Wash/System liquid.
9. It adds the Starter Kit and measures 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® Bordetella pertussis Toxin 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) 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 with LIAISON® assays should be tested, on the same specimen.

The analyzer automatically calculates *Bordetella pertussis* Toxin IgG concentrations expressed as IU/mL value and grades the results. For details, refer to the analyzer operator's manual.

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

Assay range. 10 to 140 IU/mL value *Bordetella pertussis* Toxin 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 1:5). 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 10 sample predilutions to be performed.

Dilution of over-range specimens may not provide accurate quantitative measurements due to sample-dependent unparallel response.

Sample results should be interpreted as follows:

Samples with *Bordetella pertussis* Toxin IgG concentrations below 40 IU/mL value should be graded *negative*.

Samples with *Bordetella pertussis* Toxin IgG concentrations ranging between 40 and 100 IU/mL value should be graded *Intermediate*.

Samples with *Bordetella pertussis* Toxin IgG concentrations equal to or above 100 IU/mL value should be graded *positive*.

A negative result indicates no evidence for recent contact with the pathogen. It should be underlined that the test may score negative in infected patients during early stages of infection. Combined interpretation of results with IgA is recommended.
An intermediate result indicates a possible infection. If IgA result is not available, a second sample should be collected and tested for IgG no less than two to four weeks later.
A positive result generally indicates recent exposure to the pathogen.

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

In order to achieve a comprehensive antibody profile, the following interpretation is suggested in case of combination between IgG and IgA reactivity obtained testing a single specimen. The interpretation is in accordance with the recommendations of European Reference Centres.*

IgG results	IgA results	Interpretation of results
Negative	Negative	No indication of *B. pertussis* infection
Intermediate	Negative	No recent infection
Intermediate or negative	Positive	Indication of recent infection
Positive	Negative or Positive	Indication of recent infection

*Guiso N. et al Eur J. Clin. Microbiol Infect Dis. 2011; 30: 307-3012; M. Riffelmann et al. J. Clin Microbiology. 2010; 48 (12): 4459-4463

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® Bordetella pertussis Toxin test is used in conjunction with other manufacturers' assays for detection of specific *Bordetella pertussis* Toxin 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 quantitatively for the presence of *Bordetella pertussis* Toxin 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, in association with medical judgement.

If infection is suspected in infants under the age of 6 months, culture or PCR tests are recommended, since children younger than 6 months rarely develop antibodies.

Serological assays cannot distinguish between immune responses following vaccination or natural infection. Serological diagnosis for *B. pertussis* infection must not be performed if vaccination took place less than 1 year before. After this period, consider vaccine management in order to interpret the serological results with confidence.

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® 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. 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® or LIAISON® XL).

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (potassium EDTA, 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).

Cross-reactions. The cross-reactivity study for the LIAISON® Bordetella pertussis Toxin IgG assay was designed to evaluate potential interference from antibodies to other organisms that may cause clinical symptoms similar to *Bordetella pertussis* infection (*Mycoplasma pneumoniae*, RSV, Adenovirus, Influenza A, Influenza B, parainfluenza 1, 2, 3 and *Chlamydia pneumoniae*), by other conditions that may result from atypical immune system activity (rheumatoid factor RF, antinuclear autoantibodies ANA, human anti-mouse antibodies HAMA), from antibodies to other organisms that may cause infectious diseases (*Toxoplasma gondii*, hCMV, Parvovirus B19, EBV, *Treponema pallidum*). Samples for these studies were pre-screened with another commercially available CE-marked assay for *Bordetella pertussis* Toxin IgG. Samples that were seronegative for *Bordetella pertussis* Toxin IgG 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 intermediate results
hCMV antibodies	5	0
Chlamydia pneumoniae IgG antibodies	6	0
Parvovirus B19 IgG antibodies	6	0
Parainfluenza 1,2,3 antibodies	2	0
Influenza A/B antibodies	6	0
EBV antibodies	5	0
Adenovirus antibodies	7	0
Toxoplasma gondii IgG antibodies	5	0
RSV antibodies	6	0
Rheumatoid factor (anti-Fc immunoglobulin)	6	0
Anti-nuclear antibodies (ANA)	5	0
Human anti-mouse antibodies (HAMA)	8	0
Mycoplasma pneumoniae IgG antibodies	5	0
Treponema pallidum antibodies	6	0
Total	78	0

There was no conclusive evidence of cross-reactivity observed since 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 on a LIAISON® Analyzer, to evaluate repeatability.

Repeatability	A	B	C	D
Number of determinations	20	20	20	20
Mean (IU/mL value)	66.8	93.8	105	90.8
Standard deviation	4.9	8.7	9.8	6.4
Coefficient of variation (%)	7.3	9.3	9.4	7.0
Min. value (IU/mL value)	58.1	78.5	89.6	79.1
Max. value (IU/mL value)	74.5	110	123	101

Reproducibility. Twenty replicates were performed in different days (one or two runs per day), with three different lots of integral, to evaluate reproducibility. The tests were performed in two testing sites, using one LIAISON® instrument each site.

Reproducibility - Site 1	A	B	C	D
LOT No. 01				
Number of determinations	20	20	20	20
Mean (IU/mL value)	51.4	86.0	107	75.4
Standard deviation	4.4	5.2	15.9	7.6
Coefficient of variation (%)	8.6	6.0	14.8	10.2
Min. value (IU/mL value)	44.0	73.9	70.9	55.3
Max. value (IU/mL value)	60.3	93.1	135	85.0
LOT No. 02				
Number of determinations	20	20	20	20
Mean (IU/mL value)	59.0	79.9	101	71.7
Standard deviation	5.1	6.8	8.9	5.9
Coefficient of variation (%)	8.6	8.5	8.8	8.3
Min. value (IU/mL value)	49.3	63.7	87.1	60.5
Max. value (IU/mL value)	67.4	93.1	118	79.6
LOT No. 03				
Number of determinations	20	20	20	20
Mean (IU/mL value)	51.2	80.2	105	71.4
Standard deviation	3.6	6.2	13.7	9.2
Coefficient of variation (%)	6.9	7.7	13.0	12.9
Min. value (IU/mL value)	44.8	68.0	68.1	42.9
Max. value (IU/mL value)	57.9	89.0	126	87.8

Reproducibility - Site 2	A	B	C	D
LOT No. 01				
Number of determinations	20	20	20	20
Mean (IU/mL value)	53.7	79.0	104	96.1
Standard deviation	3.9	11.8	11.8	11.3
Coefficient of variation (%)	7.3	14.9	11.4	11.8
Min. value (IU/mL value)	45.1	56.9	84.3	75.3
Max. value (IU/mL value)	61.7	109	133	114
LOT No. 02				
Number of determinations	20	20	20	20
Mean (IU/mL value)	56.0	73.4	93.8	83.4
Standard deviation	4.5	7.3	12.6	8.3
Coefficient of variation (%)	8.0	9.9	13.5	9.9
Min. value (IU/mL value)	45.2	58.1	67.0	63.9
Max. value (IU/mL value)	66.0	82.5	110	95.7
LOT No. 03				
Number of determinations	20	20	20	20
Mean (IU/mL value)	63.6	92.1	125	112
Standard deviation	4.5	10.6	13.9	12.1
Coefficient of variation (%)	7.0	11.5	11.2	10.8
Min. value (IU/mL value)	56.7	68.7	99.7	78.2
Max. value (IU/mL value)	71.4	111	140	128

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 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 on a LIAISON® XL Analyzer, to evaluate repeatability.

Repeatability	1	2	3	4
Number of determinations	20	20	20	20
Mean (IU/mL value)	60.6	87.6	108	83.5
Standard deviation	0.7	1.1	0.9	1.2
Coefficient of variation (%)	1.2	1.3	0.8	1.4
Min. value (IU/mL value)	59.1	85.0	106	81.4
Max. value (IU/mL value)	61.6	88.8	109	86.5

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) on a LIAISON® XL Analyzer, with three different lots of integral, to evaluate reproducibility.

Reproducibility - Site 1	1	2	3	4
LOT No. 01				
Number of determinations	20	20	20	20
Mean (IU/mL value)	60.2	86.7	106	77.5
Standard deviation	7.7	12.1	13.1	9.0
Coefficient of variation (%)	12.8	13.9	12.4	11.6
Min. value (IU/mL value)	42.0	54.2	72.1	50.3
Max. value (IU/mL value)	71.1	98.8	119	86.5
LOT No. 02				
Number of determinations	20	20	20	20
Mean (IU/mL value)	59.5	81.0	98.1	72.4
Standard deviation	2.6	2.9	2.7	3.8
Coefficient of variation (%)	4.4	3.6	2.8	5.3
Min. value (IU/mL value)	54.7	76.9	90.8	64.1
Max. value (IU/mL value)	64.9	88.9	102	78.7
LOT No. 03				
Number of determinations	20	20	20	20
Mean (IU/mL value)	70.6	98.7	121	88.0
Standard deviation	2.7	3.6	4.9	4.2
Coefficient of variation (%)	3.9	3.7	4.0	4.8
Min. value (IU/mL value)	66.9	92.0	114	83.2
Max. value (IU/mL value)	77.0	104	129	96.9

Reproducibility - Site 2	1	2	3	4
LOT No. 01				
Number of determinations	20	20	20	20
Mean (IU/mL value)	56.7	83.6	104	77.5
Standard deviation	4.7	6.2	7.3	5.5
Coefficient of variation (%)	8.3	7.4	7.1	7.1
Min. value (IU/mL value)	51.4	74.6	94.5	71.0
Max. value (IU/mL value)	65.0	95.9	118	85.9
LOT No. 02				
Number of determinations	20	20	20	20
Mean (IU/mL value)	51.9	71.8	86.8	66.0
Standard deviation	3.6	4.4	5.4	4.4
Coefficient of variation (%)	6.9	6.1	6.2	6.6
Min. value (IU/mL value)	47.6	66.6	80.6	60.6
Max. value (IU/mL value)	59.3	81.1	96.8	74.1
LOT No. 03				
Number of determinations	20	20	20	20
Mean (IU/mL value)	66.0	94.9	113	86.5
Standard deviation	5.1	6.8	2.8	5.6
Coefficient of variation (%)	7.6	7.2	2.5	6.5
Min. value (IU/mL value)	57.7	84.0	108	77.0
Max. value (IU/mL value)	74.9	107	119	96.9

15.4. 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 *Bordetella pertussis* Toxin IgG. All samples resulted in high concentration values as expected, indicating no sample misclassification.

15.5. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 931 specimens collected without pre-selection from routine testing of European and Australian laboratories. The specimens were tested by LIAISON® Bordetella pertussis Toxin IgG and LIAISON® Bordetella pertussis Toxin IgA. The specimens were tested in parallel with a reference CE-marked method. Consensus with additional serological data was applied to define the expected results.

54 Positive, 3 Intermediate and 3 Negative results were observed out of 60 specimens expected to be Positive which were tested during performance evaluation, while 4 Positive, 52 Intermediate and 4 Negative results were observed out of 60 specimens expected to be Intermediate.

795 Negative, 12 Intermediate and 2 Positive results were observed out of 809 specimens expected to be Negative.

After consensus, an overall concordance of 97.7% (95% CI 96.6 – 98.6%) of Liaison IgG versus the expected results was obtained.

Considering LIAISON® assays and reference methods, the following prevalence table was obtained in the study, for IgG and IgA combined results:

IgG result	IgA result	Interpretation of results	Prevalence by LIAISON® assays (CLIA)	Prevalence by reference CE-marked methods (EIA)
Negative	Negative	No indication of *B. pertussis* infection	738 (79.3%)	736 (79.1%)
Negative	Positive	Indication of recent infection	66 (7.1%)	80 (8.6%)
Intermediate	Positive	Indication of recent infection	17 (1.8%)	22 (2.4%)
Positive	Positive	Indication of recent infection	32 (3.4%)	31 (3.3%)
Positive	Negative	Indication of recent infection	28 (3.0%)	16 (1.7%)
Intermediate	Negative	No recent infection	50 (5.4%)	46 (4.9%)

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