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Deletions: -

LIAISON® Biotrin Parvovirus B19 IgG Plus (REF 311540)

1. INTENDED PURPOSE

The LIAISON® Biotrin Parvovirus B19 IgG Plus, a chemiluminescent immunoassay (CLIA), is intended for the quantitative detection of IgG antibodies to the B19 virus (B19V, previously known as human parvovirus B19) in human serum, lithium, and sodium heparin, K_2-EDTA and sodium citrated plasma. The test, in conjunction with the LIAISON® Biotrin Parvovirus B19 IgM Plus (chemiluminescent immunoassay), may be used for testing women of childbearing age to determine their serological status where there is a suspicion of exposure to B19V. The results of these assays may be used to make a serological determination of past, recent, or current infection with B19V. The test may also be used for all patients, including pregnant women and children younger than 14 years as an aid in the diagnosis of fifth disease (erythema infectiosum). The test must be performed on the LIAISON® Analyzer family*.

2. SUMMARY AND EXPLANATION OF THE TEST

Parvovirus B19 (B19) is a small, non-enveloped ssDNA erythrovirus of 20–25 nm in diameter with a 5.6 kb genome and a single promoter. Given the absence of a lipid envelope and its limited DNA content, B19 is very resistant to the routine treatment of blood products. The discovery of variants of parvovirus B19 led to a subdivision that includes now genotypes 2 and 3, strains A6/K71 and V9, respectively, in addition to genotype 1 (B19). [1-11]

Most commonly, transmission of B19 happens through personal contact, via aerosol or respiratory secretions. The virus can also be transmitted transplacentally from an infected mother to her fetus, and iatrogenically by contaminated blood products. Viral entry into target cells is mediated by cellular receptors. After initial replication, which likely occurs in the respiratory tract, the virus enters bone marrow reticulocytes and replicates there, inhibiting normal erythropoiesis. B19 is cytotoxic to erythroid progenitor cells, inducing apoptosis-like events and expression of the interleukin 6. The resulting erythropoietic stress can cause complications, especially to immunocompromised individuals and subjects with underlying hemolytic diseases. [1-11]

B19 viremia occurs one week after exposure and usually lasts about 5 days. In general, IgM antibodies to B19 appear 7-10 days after infection, and persist for approximately 3 months. Anti-B19 IgG antibodies become detectable about 15 days post-infection and increase exponentially in the subsequent days, to remain high for the rest of the individual's life, providing long-term protection. [1-11]

Infections with parvovirus B19 are very common during childhood, and it is estimated that 40-60% of adults have evidence of a history of infection, a percentage that reaches 90% in the elderly population. Despite such high prevalence, viremia is actually rare, most likely as a consequence of virus neutralization by immunoglobulins in healthy subjects. B19 causes erythema infectiosum (EI), a common mild childhood illness characterized by erythematous rashes affecting the face, trunk, and limbs. EI is also known as fifth disease, as it is the fifth most-common rash-causing illness during childhood. [2,5,6,8,12]

Infections in pregnant women risk possible transplacental viral transmission to the fetus. Although not common, this may be the cause of serious complications such as fetal anemia, neurological anomalies, hydrops fetalis, and fetal death in about 5% of transmissions[1,2,5,8,9] It is estimated that the percentage of pregnant women susceptible to B19 infection is high (30-50%), but only a minor fraction of them will actually be infected. The prevalence of seroconversion among pregnant women is 1.5-3% in normal endemic times and can rise to 10-14% during epidemics. [3,4,6,7,11]

Clinical diagnosis of B19 infections can be made based on EI, when present. When laboratory confirmation is required, or in those cases where the typical rashes of EI are not present, serologic detection of anti-B19-specific IgM and IgG antibodies is the diagnostic method of choice to diagnose parvovirus infections in immunocompetent individuals[2-10,12]. Considering the time of onset of immunoglobulins and their above-described development, serology can also provide information on the infection phase. Pregnant women showing an IgM+/IgG- profile should repeat serology in 3-4 weeks or be referred for ultrasound and standard workup against fetal complications. Detection of both IgM and IgG diagnoses a recent infection, whereas the absence of anti-B19 IgM and IgG antibodies indicates that the subject has no active infection ongoing; however, in the latter case, another serologic assessment after 2 weeks and/or PCR assessment of viral load is recommended, especially in pregnant women. A positive anti-B19 IgG result with a negative IgM result indicates immunity. Such an outcome is particularly important to reassure recently exposed pregnant women, or pregnant women with symptoms, that they are immune. Nevertheless, in rare cases, the presence of IgG antibodies without IgM may be due to premature clearance of IgM immunoglobulin and needs attention, again especially in pregnant women. [2-4,6,9,12]

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination and qualitative detection of specific IgG to parvovirus B19 is an indirect sandwich chemiluminescence immunoassay (CLIA). Recombinant parvovirus B19 VP2 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, parvovirus B19 antibodies, if present in calibrators, samples, or controls, bind to the solid phase. During the second incubation, the antibody conjugate reacts with any human anti-parvovirus B19 IgG already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of anti-VP2 IgG concentration present in calibrators, samples, or controls.

*(LIAISON® XL, LIAISON® XS)

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.45 mL)	SORB	Magnetic particles (approx. ≥ 0.375%) coated with recombinant parvovirus B19 VP2 antigen (obtained in baculovirus, approx. 10 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.0 mL)	CAL 1	Human serum/plasma containing low parvovirus B19 IgG levels (approx. 3 IU/mL), BSA, phosphate buffer, detergents, ProClin™ 300, an inert yellow dye. The calibrator concentrations (IU/mL) are referenced to the WHO 2nd International Standard for Anti-Parvovirus B19, NIBSC Code 01/602.
Calibrator 2 (2.0 mL)	CAL 2	Human serum/plasma containing high parvovirus B19 IgG levels (approx. 100 IU/mL), BSA, phosphate buffer, detergents, ProClin™ 300, an inert blue dye. The calibrator concentrations (IU/mL) are referenced to the WHO 2nd International Standard for Anti-Parvovirus B19, NIBSC Code 01/602.
Specimen diluent (2 x 28 mL)	DIL SPE	Casein, BSA, phosphate buffer, detergents, ProClin™ 300, an inert blue dye.
Conjugate (28 mL)	CONJ	Mouse monoclonal IgG antibodies to human IgG conjugated to an isoluminol derivative (minimum 10 ng/mL), BSA, phosphate buffer, ProClin™ 300, preservatives.
Number of tests		100

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

Materials required but not provided (system related)

LIAISON® XL Analyzer	**LIAISON® XS Analyzer**
LIAISON® XL Cuvettes (REF X0016).	LIAISON® Cuvettes on Tray (REF X0053).
LIAISON® XL Disposable Tips (REF X0015) or	LIAISON® Disposable Tips (REF X0055).
LIAISON® Disposable Tips (REF X0055).	–
LIAISON® XL Starter Kit (REF 319200) or	LIAISON® EASY Starter Kit (REF 319300).
LIAISON® EASY Starter Kit (REF 319300).	–
LIAISON® Wash/System Liquid (REF 319100).	LIAISON® EASY Wash Buffer (REF 319301).
–	LIAISON® EASY System Liquid (REF 319302).
LIAISON® XL Waste Bags (REF X0025).	LIAISON® EASY Waste (REF X0054).
–	LIAISON® EASY Cleaning Tool (REF 310996).

Additional required materials

LIAISON® Biotrin Control Parvovirus B19 IgG Plus (negative and positive) (REF 311541).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

For Laboratory Professional Use Only.

All serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1 and anti-HIV-2 and were found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

Visually inspect the integral vials for leakage at the membrane seals or elsewhere. If the vials are found to be leaking, the local customer service should be notified immediately.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics during the assay.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents; the waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each country.

Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Pursuant to EC Regulation 1272/2008 (CLP), hazardous reagents are classified and labeled as follows:

REAGENTS:	CAL 1, CAL 2, DIL SPE, CONJ
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+P364 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labeled as EUH210 safety data sheets available on request.

For additional information see the Safety Data Sheets available on www.diasorin.com.

7. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension. Before the seal is removed, rotate the small wheel in the magnetic particle compartment until the color of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles are resuspended. Carefully wipe the surface of each septum to remove residual liquid. Repeat as necessary until the magnetic particles are completely resuspended. Incomplete magnetic particle resuspension may cause variable and inaccurate analytical results.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

Visually inspect the reagents, and the 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® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device, which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
  a. Insert the reagent integral into the dedicated slot.
  b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before use. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.
- Visually inspect the vials for leaking. If the vials are found to be leaking, the local customer service should be notified

8. STORAGE AND STABILITY OF THE REAGENT INTEGRAL

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

9. SPECIMEN COLLECTION AND PREPARATION

The correct specimen type must be used in the assay. The following matrices have been tested and may be used:
– Serum (without and with gel- SST);
– Sodium citrate plasma;
– Sodium and lithium heparin plasma;
– K_2-EDTA plasma.

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

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

Package and label specimens must be in compliance with applicable state, federal, and international regulations covering the transportation 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:
– 15°-30°C for three (3) days, in any case, room temperature storage should be avoided;
– 2°-8°C for three (3) days, otherwise they should be aliquoted and stored deep-frozen (–20°C or below);
– Up to six (6) freeze-thaw cycles, however multiple freeze-thaw cycles should be avoided;
– Up to three (3) months at -20°C or below.

If samples are stored frozen, mix thawed samples well before testing.

Further centrifugation of specimens removed from red cells, clot, or gel separator (preferably 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 hemolyzed 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

Testing of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve.

Each calibration solution allows five (5) calibrations to be performed.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:
– A new lot of reagent integral or of Starter Kit is used.
– The previous calibration was performed more than eight (8) weeks before.
– Control values lie outside the expected ranges.
– **LIAISON® XL Analyzer**: the analyzer has been serviced.
– **LIAISON® XS Analyzer**: after a technical intervention, only if required by the service procedure, as communicated by DiaSorin Technical support or representative.

LIAISON® XL and LIAISON® XS analyzers: 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.

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 instructions.

The analyzer operations are as follows:
1. Dispense specimen (calibrator or control), coated magnetic particles, specimen diluent into the reaction cuvettes
2. Incubate and wash
3. Dispense the Conjugate into the reaction cuvettes
4. Incubate and wash
5. Add the Starter Reagents 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® Biotrin Control Parvovirus B19 IgG Plus (REF 311541)

(a) at least once per day of use,

(b) whenever the kit is calibrated,

(c) whenever a new lot of Starter Reagents is used, 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 parvovirus B19 IgG antibody concentrations expressed as IU/mL and grades the results. For details, refer to the analyzer operator's manual.

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

Assay range. 0.1 to 150 IU/mL parvovirus B19 IgG antibodies.

Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:10). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows at least 20 sample predilutions to be performed.

Sample results should be interpreted as follows:

LIAISON® Biotrin Parvovirus B19 IgG Plus		
IU/mL	**Results**	**Rules and interpretation**
< 2.00	Negative	A result below 2.00 IU/mL may indicate the absence, or a level of IgG antibodies to parvovirus B19 below the threshold.
2.00 – 2.49	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 week later when the result is repeatedly equivocal.
≥ 2.50	Positive	A result above or equal to 2.50 IU/mL generally indicates exposure of the subject to parvovirus B19.

A negative result for IgG antibodies to parvovirus B19 generally indicates that the patient has not been infected but does not exclude the possibility of acute parvovirus B19 infection, because the infection may be in its very early stage and the patient may be still unable to synthesize parvovirus B19 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 parvovirus B19 is suspected despite a negative or equivocal finding, a second sample should be collected and tested for IgM and IgG during the course of infection.

A positive result for IgG antibodies to parvovirus B19 generally indicates a previous infection 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® Biotrin Parvovirus B19 IgG Plus test is used in conjunction with other manufacturers' assays for the detection of specific parvovirus B19 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.
– Performance has not been established for the use of bodily fluids other than human ones.
– The combination of LIAISON® IgM and IgG test and clinical data is recommended when the diagnosis of parvovirus B19 is based on a single specimen. A single result may not be sufficient for diagnosis but should be determined in conjunction with clinical findings, patient history and always in association with medical judgment.
– Results obtained with LIAISON® Biotrin Parvovirus B19 IgG Plus assay may not be used interchangeably with values obtained using different manufacturers' assay methods.
– 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.

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, hemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference.

Controlled studies of potentially interfering substances showed no interference to each substance listed below in the LIAISON® Biotrin Parvovirus B19 IgG Plus, at the indicated concentration.

Substances	Tested concentrations
Triglycerides	1500 mg/dL
Hemoglobin	1000 mg/dL
Unconjugated bilirubin	40 mg/dL
Conjugated bilirubin	40 mg/dL
Biotin	3500 ng/mL
Human Albumin	6000 mg/dL
Cholesterol	400 mg/dL
Total human IgG	2000 mg/dL
Total human IgM	400 mg/dL
Total Protein (high)	120 g/L
Total Protein (low)	60 g/L
Vitamin A	800 µg/dL
Vitamin B12	2850 pg/mL
Vitamin C	20 mg/dL
Vitamin D	450 ng/mL
Vitamin E	120 mg/L
Folic Acid	160 ng/mL
Acetaminophen	15.6 mg/dL
Ibuprofen	21.9 mg/dL
Naproxen	36.0 mg/dL
Penicillin G	110 mg/dL
Streptomycin (sulphate)	25.8 mg/dL
Erythromycin	13.8 mg/dL

Cross-reactions.

The cross-reactivity study for the LIAISON® Biotrin Parvovirus B19 IgG Plus assay was designed to evaluate potential interference from antibodies to other viruses that may cause infectious diseases, as well as from other conditions. Samples for these studies were pre-screened with another commercially available parvovirus B19 IgG assay. If found negative for parvovirus B19 IgG antibodies, those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays. The observed specificity for potentially cross-reactive specimens is comparable to that of open populations.

Condition	Number of tested samples	Assay Positive results
CMV (anti-CMV IgG and/or IgM positive)	13	0
Epstein-Bar Virus (anti-EBV IgG and/or IgM positive)	12	0
Herpes Simplex Virus (anti-HSV IgG and/or IgM positive)	13	0
Rubella (anti-Rubella IgG and/or IgM positive)	12	0
Hepatitis C virus (anti-HCV positive)	9	0
Human Immunodeficiency Virus (anti-HIV antibodies)	9	0
Hepatitis A virus (anti-HAV IgG and/or IgM positive)	12	0
Borrelia burgdorferi (*anti-B. burgdorferi IgG* and/or IgM antibodies)	11	0
Toxoplasma gondii (*anti-T. gondii IgG* and/or IgM antibodies)	7	0
Varicella Zoster Virus (anti-VZV IgG and/or IgM positive)	16	0
Measles virus (anti-Measles IgG and/or IgM positive antibodies)	12	0
Mumps virus (anti-Mumps IgG and/or IgM positive antibodies)	11	0
Adenovirus (anti-Adenovirus antibodies)	14	0
Anti-Influenza A antibodies	9	0
Anti-Influenza B antibodies	8	0
Parainfluenza antibodies	1	0
Respiratory syncytial virus (RSV) antibodies	9	0
Mycoplasma pneumonia (*anti-M. pneumonia IgG* and/or IgM positive)	14	0
Treponema Pallidum (*anti-T. pallidum* antibodies)	14	0
Rheumatoid Factor (anti-Fc Immunoglobulin)	7	0
Human anti-mouse antibodies (HAMA)	8	0
Anti-nuclear antibodies (ANA)	17	0
Total	**238**	**0**

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® XL Analyzer

A twenty-day precision study was performed in accordance with CLSI document EP5-A3, using a coded panel of six (6) samples prepared by either spiking or diluting samples as necessary to obtain negative, low positive and positive samples. Kit Controls set was also included in the study. The panel samples and kit controls were tested with the LIAISON® Biotrin Parvovirus B19 IgG Plus assay in two (2) replicates per run, two (2) runs per day for twenty (20) operating days on one LIAISON® XL Analyzer, on three (3) assay lots.

Sample ID	N	Mean IU/mL	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative Control A	240	515(*)	45.29	8.8%	40.23	7.8%	70.97	13.8%	62.62	12.2%	112.37	21.8%
Positive Control A	240	4.53	0.13	2.9%	0.19	4.1%	0.33	7.2%	0.10	2.2%	0.41	9.1%
Sample 1	240	0.640	0.03	4.4%	0.02	3.7%	0.03	4.8%	0.06	9.3%	0.08	11.9%
Sample 2	240	1.79	0.05	2.6%	0.03	1.6%	0.07	3.8%	0.08	4.7%	0.12	6.7%
Sample 3	240	2.66	0.05	1.9%	0.05	1.9%	0.11	4.0%	0.18	6.8%	0.22	8.4%
Sample 4	240	7.22	0.21	2.9%	0.18	2.4%	0.24	3.3%	0.16	2.1%	0.40	5.5%
Sample 5	240	8.32	0.19	2.3%	0.19	2.3%	0.32	3.9%	0.23	2.7%	0.48	5.8%
Sample 6	240	67.6	2.88	4.3%	2.47	3.7%	5.75	8.5%	2.39	3.5%	7.29	10.8%

* Elaboration performed on RLU value.

15.3. Precision with LIAISON® XS Analyzer

A twenty-day precision study was performed in accordance with CLSI document EP5-A3, using a coded panel of six (6) samples prepared by either spiking or diluting samples as necessary to obtain negative, low positive and positive samples. Kit Controls set was also included in the study. The panel samples and kit controls were tested with the LIAISON® Biotrin Parvovirus B19 IgG Plus assay in two (2) replicates per run, two (2) runs per day for twenty (20) operating days on one LIAISON® XS Analyzer, on three (3) assay lots.

Sample ID	N	Mean IU/mL	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative Control A	240	400(*)	16.88	4.2%	39.85	10.0%	50.01	12.5%	34.56	8.6%	74.62	18.7%
Positive Control A	240	4.73	0.12	2.6%	0.19	4.0%	0.60	12.7%	0.06	1.3%	0.64	13.6%
Sample 1	240	0.665	0.02	2.5%	0.02	2.4%	0.07	10.3%	0.04	5.3%	0.08	12.1%
Sample 2	240	1.91	0.03	1.6%	0.03	1.6%	0.12	6.1%	0.09	4.9%	0.16	8.1%
Sample 3	240	2.87	0.05	1.8%	0.06	2.1%	0.18	6.3%	0.12	4.2%	0.23	8.1%
Sample 4	240	6.95	0.16	2.3%	0.16	2.3%	0.38	5.5%	0.29	4.2%	0.53	7.6%
Sample 5	240	8.98	0.28	3.1%	0.15	1.6%	0.57	6.3%	0.46	5.2%	0.80	8.9%
Sample 6	240	71.7	4.39	6.1%	3.85	5.4%	7.37	10.3%	0.00	0.0%	9.40	13.1%

* Elaboration performed on RLU value.

15.4. Linearity and Trueness

The assay linearity was checked by the dilution test.

Dilution test. Four samples containing parvovirus IgG concentrations were tested as such and after serially diluting with specimen diluent or according to the matrix of origin (serum or plasmas). Parvovirus IgG concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) were all above 0.96, with slope within 0.9 – 1.1.

Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery
>150	>150	–	>150	>150	–
126	126	100%	135	135	100%
63.0	62.7	100%	67.5	63.0	93%
31.5	29.6	94%	33.8	31.9	95%
15.8	15.7	100%	16.9	15.3	91%
7.88	7.32	93%	8.44	7.51	89%
3.94	3.65	93%	4.22	3.45	82%
1.97	1.91	97%	2.11	1.74	82%

Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery	Expected concentration (IU/mL)	Measured concentration (IU/mL)	% Recovery
78.0	78.0	100%	78.0	78.0	100%
39.0	40.0	103%	39.0	39.9	102%
19.5	20.2	104%	19.5	20.1	103%
9.75	9.95	102%	9.75	10.2	105%
4.88	4.75	97%	4.88	5.11	105%
2.44	2.48	102%	2.44	2.50	103%
1.22	1.17	96%	1.22	1.32	108%

The assay trueness was checked by dilution test of WHO standard (NIBSC Code 01/602). Slope was found within range 0.95 – 1.05 with correlation coefficients (r) equal to 1.00.

15.5. Analytical Sensitivity as Seroconversion Panel Performance

Eight (8) commercially available Parvovirus B19 seroconversion panels were tested using LIAISON® Biotrin Parvovirus B19 IgG Plus and a commercially available CE-marked Parvovirus B19 IgG comparator assay, to determine the sensitivity of the assay. The results are summarized in the following table:

Panel ID	LIAISON® Biotrin Parvovirus B19 IgG Plus		Parvovirus B19 IgG Comparator assay	
	Last day with negative results	First day with positive results	Last day with negative results	First day with positive results
Panel 1	8	11	8	11
Panel 2	14	n.a.	14	n.a.
Panel 3	n.a.	0	n.a.	0
Panel 4	0	9	0	9
Panel 5	0	4	0	4
Panel 6	5	7	7	n.a.
Panel 7	4	12	4	12
Panel 8	7	14	11	14

15.6. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than the real ones. 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 diluting three high-titred samples positive for parvovirus IgG. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification and with no high-dose saturation effect observed.

15.7. Analytical and functional sensitivity

Analytical sensitivity is defined as the minimum detectable dose distinguishable from zero by 1.649 standard deviations. Following the method in CLSI EP17-A2, the limit of detection (LoD) for the LIAISON® Biotrin Parvovirus B19 IgG Plus assay is 0.1 IU/mL. Functional sensitivity is defined as the lowest analyte concentration that can be determined with an inter-assay CV < 20%. Following the method from CLSI EP17-A2, the limit of quantitation (LoQ) for the LIAISON® Biotrin Parvovirus B19 IgG Plus assay equal to or lower than 0.122 IU/mL.

15.8. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 1471 either selected or unselected specimens collected from several European laboratories. Different groups of subjects were tested including women of childbearing age, pregnant women, adult males and children younger than 14 years. The specimens were tested using a comparison method and discordant or equivocal findings were resolved by a third method to define the expected results.

Two positive, one equivocal and 520 negative results were observed in the expected negative population studied - diagnostic specificity: 99.43% (520/523) (95% confidence interval: 98.3-99.8%).

Two equivocal and 946 positive results were observed in the expected positive population studied - diagnostic sensitivity: 99.79% (946/948) (95% confidence interval: 99.2-99.9%).

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 the Competent Authority of the EU Member State in which the user and/or the patient is established.

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