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CE 0459

LIAISON® VZV IgG HT (REF 311850)

1. INTENDED PURPOSE

The LIAISON® VZV IgG HT assay uses chemiluminescent immunoassay (CLIA) technology for the in vitro quantitative determination of specific IgG antibodies to varicella-zoster virus (VZV) in human serum, lithium and sodium heparin and K_2-EDTA plasma. This assay is intended as an aid in the determination of immune status to VZV and as an aid in the diagnosis of infection of varicella-zoster virus in individuals including pregnant women, transplanted and immunocompromised. The test has to be performed on the LIAISON® XL and LIAISON® XS analyzers only.

2. SUMMARY AND EXPLANATION OF THE TEST

The varicella-zoster virus (VZV) is a neurotropic human-restricted alpha-herpes virus of the Varicella genus, of which the main characteristic is the capacity for latency.[1,2] The VZV genome is a single double-stranded 125-kb DNA molecule containing 71 open reading frames and packaged into an icosahedral nucleocapsid. A tegument layer surrounds the nucleocapsid and is encapsulated by a host-derived lipid envelope containing viral glycoproteins.[2] Among several polypeptides encoded by the VZV genome, glycoprotein B is essential for infectivity and is the major target of neutralizing antibodies. Glycoprotein E is the most abundant and highly immunogenic.[3]

VZV is the etiological agent of varicella, also known as chickenpox, and herpes zoster, also known as shingles.[4] Varicella is the manifestation of a primary VZV infection in patients without prior exposure, usually in childhood.[1,5] In previously healthy children, varicella is acute, self-limiting and rarely causes death. However, morbidity is high and may be associated with several complications.[6]

Systemic dissemination of the virus via infected T cells results in diffuse vesicular lesions on the trunk, head and extremities, characteristic of varicella.[1,5] Severe varicella in children occurs rarely and involves secondary bacterial skin and soft tissue infections or complications in the central nervous system.[2] During the initial infection, virions are transported along sensory nerves from the skin to establish a reservoir of latent VZV in cranial nerve ganglia, dorsal root ganglia and autonomic ganglia.[1] Reactivation of the latent infection results in herpes zoster, and its most prominent feature is a painful unilateral vesicular lesion in a dermatomal region, although a rash-free clinical manifestation can also occur.[4,5]

Primary infection induces VZV-specific antibodies and T cell-mediated immune responses, which further protect against secondary infections.[7] This response persists for life in immunocompetent individuals.[7] VZV antibody levels do not decline over time, but T cell-mediated immunity decreases with increasing age and in the presence of immunosuppressive diseases.[7,8] As a result, VZV reactivation may occur in the form of herpes zoster.[7,9]

Within the European Union, few member states have implemented recommendations for universal vaccination.[10] It is estimated that in the absence of universal vaccination, 5.5 million cases of varicella occur annually in the European Union, with 54% resulting in an ambulatory primary care visit and 0.3% requiring hospitalization.[10] Vaccination results in the appearance of VZV-specific antibodies and a T cell-mediated immune response.[2,7] In 15% of vaccinees, a mild form of varicella will still appear due to primary vaccine failure.[7]

Varicella is highly transmissible and present in all countries. In the absence of immunization, at least 90% of the population acquire the virus within the first 15 years of age in countries with a temperate climate. There is a greater risk of herpes zoster development in immunocompromised patients and individuals over the age of 50, while it is uncommon in children[11,12].

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgG to varicella-zoster virus is an indirect chemiluminescence immunoassay (CLIA). The varicella-zoster virus antigen is used for coating magnetic particles (solid phase) and mouse monoclonal antibody directed against human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, anti-VZV 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-VZV 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-VZV IgG concentration present in calibrators, samples or controls.

4. MATERIALS PROVIDED

Reagent integral

Magnetic particles (2.45 mL)	SORB	Magnetic particles (approx. ≥ 0.25%) coated with varicella-zoster virus glycoprotein antigen (partially purified extract of infected cell cultures, Ellen strain, approx. 50 µg/mL), BSA, phosphate buffer, < 0.1% sodium azide.
Calibrator 1 (2.1 mL)	CAL 1	Human serum/plasma containing low varicella-zoster virus IgG levels (approx. 190 mIU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (mIU/mL) are referenced to the WHO 1st International Standard Preparation, 1987 (W1044).
Calibrator 2 (2.1 mL)	CAL 2	Human serum/plasma containing high varicella-zoster virus IgG levels (approx. 1950 mIU/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (mIU/mL) are referenced to the WHO 1st International Standard Preparation, 1987 (W1044).
Specimen diluent (4.5 mL)	DIL SPE	Casein, BSA, phosphate buffer, detergents, 0.2% ProClin™ 300, preservatives.
Assay Buffer (2 x 28.5 mL)	BUF	Casein, BSA, phosphate buffer, detergents, 0.2% ProClin™ 300, preservatives, an inert blue dye.
Conjugate (28.5 mL)	CONJ	Mouse monoclonal IgG antibodies to human IgG conjugated to an isoluminol derivative (minimum 100 ng/mL), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives.
Number of tests		200

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® Control VZV IgG HT (negative and positive) (REF 311851).

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. 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.

The LIAISON® XL and LIAISON® XS analyzers should be cleaned and decontaminated on a regular basis. See the Operator's Manual for the procedures.

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

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

REAGENTS:	CAL 1, CAL 2, BUF, DIL SPE, CONJ
CLASSIFICATION:	Skin sens. 1A H317 Aquatic chronic 3 H412
SIGNAL WORD:	Warning
SYMBOLS / PICTOGRAMS:	 GHS07 Exclamation mark
HAZARD STATEMENTS:	H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects.
PRECAUTIONARY STATEMENTS:	P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse.
CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008).	reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300).

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

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

7. PREPARATION OF THE REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

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

Foaming of reagents

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

Visually inspect the reagents, 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 expiration date.
- **Opened on board or at 2-8°C**: up to eight (8) weeks.
- 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 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 regulations covering the transportation of clinical specimens and infectious substances.

Specimens may be shipped on dry ice (frozen) or 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 that 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 seven (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;
– Up to one (1) month 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, lipemia 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 lipemic 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 the DiaSorin Technical support team or a 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 team 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® Control VZV IgG HT (REF 311851)

(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 with the 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 VZV IgG antibody concentrations expressed as mIU/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.

Standardization

The LIAISON® VZV IgG HT assay is standardized against the WHO 1st International Standard Preparation, 1987 (W1044).

Assay range. 10 to 4000 mIU/mL VZV 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 in the reagent integral allows at least 30 sample predilutions to be performed.

Sample results should be interpreted as follows:

LIAISON® VZV IgG HT		
mIU/mL	**Results**	**Rules and interpretation**
< 150.0	Negative	A result below 150 mIU/mL may indicate the absence or a level of IgG antibodies to VZV below the threshold.
≥ 150.0	Positive	A result above or equal to 150 mIU/mL generally indicates exposure of the subject to VZV.

Assay file for VZV IgG determination with the above interpretation of results is **VZGH150**.

A negative result for IgG antibodies to VZV generally indicates that immunity has not been acquired, but does not exclude the possibility of acute VZV infection, because the infection may be in its very early stage and the patient may be still unable to synthesize VZV specific antibodies or the antibodies may be present in undetectable levels. If exposure to varicella-zoster virus is suspected despite a negative 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 VZV generally indicates exposure to the pathogen or administration of immunoglobulins, but it is no indication of active infection or stage of disease.

Note - The German Permanent Commission for Vaccination (Impfempfehlungen der Ständigen Impfkommission, STIKO) suggests adopting the following interpretation of results in Epidemiologisches Bulletin No. 8 (Robert-Koch-Institut, 2001, Berlin, Germany):

Samples with varicella-zoster virus IgG concentrations below 50 mIU/mL should be graded *negative*.

Samples with varicella-zoster virus IgG concentrations ranging between 50 and 100 mIU/mL should be graded borderline, but results are to be considered *negative*.

Samples with varicella-zoster virus IgG concentrations above 100 mIU/mL should be graded *positive*.

To adopt the option at 100 mIU/mL, please use assay file **VZGH100**.

Specific performance characteristics are provided in paragraph 15.8 for both interpretations of results.

14. LIMITATIONS OF THE PROCEDURE

Assay performance characteristics have not been established when any LIAISON® VZV IgG HT test is used in conjunction with other manufacturers' assays for the detection of specific varicella-zoster virus 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 varicella-zoster virus 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.
– Dose values obtained using different manufacturers' assay methods may not be interchangeable.
– 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 analytes in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, hemolysis, effects of sample treatment) or cross-reactive antibodies.

Interference

Controlled studies of potentially interfering substances showed no interference with each substance listed below in the LIAISON® VZV IgG HT, at the indicated concentration.

Substances	Tested concentrations	Substances	Tested concentrations
Triglycerides	3000 mg/dL	Vitamin B12	2850 pg/mL
Hemoglobin	1000 mg/dL	Vitamin C	20 mg/dL
Unconjugated bilirubin	40 mg/dL	Vitamin D	450 ng/mL
Conjugated bilirubin	40 mg/dL	Vitamin E	120 mg/L
Biotin	3500 ng/mL	Folic Acid	160 ng/mL
Human Albumin	6000 mg/dL	Acetaminophen	15.6 mg/dL
Cholesterol	400 mg/dL	Ibuprofen	21.9 mg/dL
Total human IgG	2000 mg/dL	Acetylsalicylic acid	50 mg/dL
Total human IgM	400 mg/dL	Naproxen	36.0 mg/dL
Total Protein (high)	120 g/L	Penicillin	110 mg/dL
Total Protein (low)	60 g/L	Streptomycin (sulphate)	25.8 mg/dL
Vitamin A	800 µg/dL	Erythromycin	13.8 mg/dL

Cross-reactions

The cross-reactivity study for the LIAISON® VZV IgG HT 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 VZV IgG assay. If found negative for VZV 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 Reactive results
CMV (anti-CMV positive)	8	0
Epstein-Barr Virus (anti-EBV positive)	8	0
Herpes Simplex Virus (anti-HSV 1 positive)	6	0
Herpes Simplex Virus (anti-HSV 2 positive)	10	0
Rubella (anti-Rubella positive)	7	0
Hepatitis C Virus (anti-HCV positive)	6	0
Human Immunodeficiency Virus (anti-HIV antibodies)	5	0
Hepatitis A Virus (anti-HAV positive)	5	0
Borrelia burgdorferi (anti-*B. burgdorferi* antibodies)	5	0
Toxoplasma gondii (anti-*T. gondii* antibodies)	6	0
Parvovirus B19 (anti-Parvovirus B19 positive)	16	0
Measles virus (anti-Measles antibodies)	6	0
Mumps virus (anti-Mumps antibodies)	12	0
Adenovirus (anti-Adenovirus antibodies)	6	0
Anti-Influenza A antibodies	6	0
Anti-Influenza B antibodies	5	0
Mycoplasma pneumonia (anti-*M. pneumonia* antibodies)	6	0
Respiratory syncytial virus (RSV) antibodies	7	0
Treponema Pallidum (anti-*T. pallidum* antibodies)	5	0
Rheumatoid Factor (anti-Fc Immunoglobulin)	5	0
Human anti-mouse antibodies (HAMA)	5	0
Anti-nuclear antibodies (ANA)	5	0
Total	**150**	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 seven (7) samples prepared by either spiking or diluting samples as necessary to obtain negative, near cut-off, low positive and positive samples. The Kit Controls set was also included in the study. The panel samples and kit controls were tested with the LIAISON® VZV IgG HT 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 mIU/mL	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative Control A	240	716(*)	54.1	7.5%	72.9	10.2%	95.8	13.4%	119.2	16.7%	162.4	22.7%
Negative Control B	240	646(*)	81.2	12.6%	64.4	10.0%	86.1	13.3%	115.6	17.9%	163.1	25.2%
Positive Control A	240	592.1	12.6	2.1%	14.4	2.4%	39.0	6.6%	18.4	3.1%	45.2	7.6%
Positive Control B	240	562.5	14.4	2.6%	16.5	2.9%	39.3	7.0%	16.3	2.9%	46.1	8.2%
Positive Control C	240	548.8	12.7	2.3%	14.2	2.6%	35.8	6.5%	20.3	3.7%	43.1	7.9%
Sample 1	240	18.13	1.14	6.3%	0.87	4.8%	1.32	7.3%	1.96	10.8%	2.50	13.8%
Sample 2	240	106.1	2.62	2.5%	1.66	1.6%	3.91	3.7%	2.07	2.0%	5.19	4.9%
Sample 3	240	130.1	2.58	2.0%	2.76	2.1%	4.85	3.7%	12.31	9.5%	11.74	9.0%
Sample 4	240	184.7	4.07	2.2%	2.89	1.6%	7.53	4.1%	10.09	5.5%	12.12	6.6%
Sample 5	240	520.5	12.59	2.4%	9.66	1.9%	26.45	5.1%	8.63	1.7%	31.08	6.0%
Sample 6	240	1321	36.13	2.7%	50.64	3.8%	70.30	5.3%	121.31	9.2%	135.54	10.3%
Sample 7	240	3475	114.3	3.3%	198.2	5.7%	112.2	3.2%	89.8	2.6%	262.3	7.5%

* Elaboration performed on RLU value since below the Assay range (<10 mIU/mL).

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 seven (7) samples prepared by either spiking or diluting samples as necessary to obtain negative, near cut-off, low positive and positive samples. The Kit Controls set was also included in the study. The panel samples and kit controls were tested with the LIAISON® VZV IgG HT 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 mIU/mL	Repeatability		Between Run		Between Day		Between-Lot		Total	
			SD	CV%	SD	CV%	SD	CV%	SD	CV%	SD	CV%
Negative Control A	240	808(*)	152.7	18.9%	82.6	10.2%	207.8	25.7%	98.1	12.1%	278.2	34.4%
Negative Control B	240	737(*)	126.3	17.1%	8.8	1.2%	223.8	30.4%	77.2	10.5%	260.1	35.3%
Positive Control A	240	615.3	17.0	2.8%	21.4	3.5%	29.8	4.8%	12.3	2.0%	41.1	6.7%
Positive Control B	240	564.6	15.4	2.7%	9.3	1.6%	28.7	5.1%	5.0	0.9%	33.5	5.9%
Positive Control C	240	565.1	15.9	2.8%	19.3	3.4%	20.0	3.5%	9.4	1.7%	32.5	5.8%
Sample 1	240	20.36	1.40	6.9%	1.27	6.2%	1.38	6.8%	0.86	4.2%	2.42	11.9%
Sample 2	240	107.1	2.57	2.4%	2.64	2.5%	2.39	2.2%	2.94	2.8%	4.96	4.6%
Sample 3	240	130.0	3.02	2.3%	3.28	2.5%	2.86	2.2%	10.57	8.1%	10.11	7.8%
Sample 4	240	186.8	3.92	2.1%	6.11	3.3%	5.09	2.7%	11.98	6.4%	13.14	7.0%
Sample 5	240	508.5	14.59	2.9%	15.13	3.0%	17.83	3.5%	25.15	4.9%	34.09	6.7%
Sample 6	240	1344	53.32	4.0%	41.09	3.1%	59.65	4.4%	140.39	10.4%	145.13	10.8%
Sample 7	240	3541	140.6	4.0%	178.5	5.0%	124.1	3.5%	67.2	1.9%	261.7	7.4%

* Elaboration performed on RLU value since below the Assay range (<10 mIU/mL).

15.4. Linearity

Linearity was evaluated according to CLSI EP6-A. Six (6) serum or plasma samples containing high VZV IgG concentrations were tested neat and after serially diluting with a specimen diluent or according to the matrix of origin (serum or plasma). VZV IgG concentrations measured versus concentrations expected were analyzed by linear regression.

The correlation coefficients (r) were all above 0.997, with slope within 0.9984 – 1.006.

The assay demonstrated linearity from 10 to 4000 mIU/mL.

15.5. Trueness

The assay trueness was checked on three (3) lots by means of the dilution test of WHO 1st International Standard Preparation, 1987 (W1044). The correlation coefficients (r) were all above 0.998, with slope within 0.902 – 1.058.

LIAISON® VZV IgG HT Lot A		LIAISON® VZV IgG HT Lot B		LIAISON® VZV IgG HT Lot C	
Expected concentration (mIU/mL)	**Measured concentration (mIU/mL)**	**Expected concentration (mIU/mL)**	**Measured concentration (mIU/mL)**	**Expected concentration (mIU/mL)**	**Measured concentration (mIU/mL)**
5000	>4000	5000	>4000	5000	>4000
2500	2650	2500	2561	2500	2229
1250	1248	1250	1302	1250	1234
625.0	648.9	625.0	671.1	625.0	620.0
312.5	288.4	312.5	322.8	312.5	293.7
156.3	141.1	156.3	155.8	156.3	156.1
78.13	72.23	78.13	81.26	78.13	84.0
39.06	38.88	39.06	43.74	39.06	32.30
19.53	14.98	19.53	17.03	19.53	21.8
0	<10	0	<10	0	<10
Slope: 1.058 *R: 1.00*		Slope: 1.027 R: 1.00		Slope: 0.902 R: 1.00	

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).

An analysis of the saturation effect was evaluated by diluting three high-titer samples positive for VZV IgG. All samples resulted in concentration values above the assay range that would be expected with high-titer sera, indicating no sample misclassification and with no high-dose saturation effect observed.

15.7. Limit of Blank (LoB)*

Following the method from CLSI EP17-A2, the limit of blank for the LIAISON® VZV IgG HT assay is 0.4856 mIU/mL.

*Limit of Blank, or the highest value likely to be observed with a sample containing no analyte, replaces the term “analytical sensitivity”.

15.8. Limit of Detection (LoD)

Following the method from CLSI EP17-A2, the limit of detection for the LIAISON® VZV IgG HT assay is 1.711 mIU/mL.

15.9. Limit of Quantification (LoQ)

Following the method from CLSI EP17-A2, the limit of quantification for the LIAISON® VZV IgG HT assay is 4.880 mIU/mL (defined as the lowest analyte concentration that can be determined with an inter-assay CV <20%).

15.10. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 1,549 specimens, either selected or unselected, collected from several sites. Different groups of subjects were tested including negative individuals, pregnant women, vaccinated individuals, transplant patients, immunocompromised individuals, and children younger than 14 years of age. The specimens were tested by comparison and discordant findings were resolved using a third method, by evaluating them according to the respective Instructions for Use, to define the expected results.

Two-hundred and twenty-two (222) negative results were observed in the expected negative population studied - diagnostic specificity: 100% (222/222) (95% confidence interval: 98.3-100%).

Thirty-four (34) negative and 1293 positive results were observed in the expected positive population studied - diagnostic sensitivity: 97.44% (1293/1327) (95% confidence interval: 96.4-98.2%).

According to UK Guidelines on-post exposure prophylaxis (PEP) for varicella/shingles (January 2023), a cut-off of 150 mIU/mL is recommended for immunosuppressed patients.

Diagnostic sensitivity in immunocompromised and transplant patients is 97.79% (177/181) (95% confidence interval: 94.5-99.1%).

The above results were obtained using the prescribed cut-off value at 150 mIU/mL.

Data were also evaluated using the STIKO recommendation to define the expected result (for additional information, refer to the Note in paragraph 13).

If diagnostic specificity and sensitivity are calculated in the same population using the cut-off value at 100 mIU/mL, the following results are then obtained.

Diagnostic specificity: 99.50% (199/200) - 95% confidence interval: 97.2-99.9%.

Diagnostic sensitivity: 99.11% (1337/1349) - 95% confidence interval: 98.5-99.5%.

According to UK Guidelines on post-exposure prophylaxis (PEP) for varicella/shingles (January 2023), a cut-off of 100 mIU/mL is recommended for pregnant women.
Diagnostic sensitivity in pregnant women is 98.85% (86/87) (95% confidence interval: 93.8-99.8%).

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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