VZV IgG

Purpose

This procedure provides instructions for performing VZV IgG on the DIASORIN LIAISON.

Policy Statements

This procedure applies to all laboratory technical staff responsible for performing VZV IgG testing on the DiaSorin Liaison.

Principle

The DiaSorin LIAISON® VZV IgG uses chemiluminescence immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative detection of specific IgG antibodies to varicella-zoster virus (VZV) in human serum. This assay can be used as an aid in the determination of previous infection of varicella-zoster virus.

The method for the qualitative determination of specific IgG to varicella-zoster virus is an indirect chemiluminescence immunoassay (CLIA). All assay steps and incubations are performed by the LIAISON® Analyzer family. Varicella-zoster virus antigen is used for coating magnetic particles (solid phase) and a mouse monoclonal antibody to human IgG is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, anti-VZV IgG antibodies, present in calibrators, samples or controls, bind to the solid phase. After incubation, the unbound material is removed with a wash cycle. During the second incubation, the antibody conjugate reacts with anti-VZV IgG already bound to the solid phase. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is induced. The light signal, and the amount to isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of anti-VZV IgG concentration present in calibrators, samples or controls.

Clinical Significance

Varicella (chicken pox) is an acute, highly contagious viral disease with worldwide distribution, seasonal prevalence in winter and spring, characterized by generalized vesicular exanthema often accompanied by fever. While mostly a mild disorder in childhood, varicella tends to be more severe in adults and it may be fatal, especially in neonates and in immunocompromised subjects.

Varicella-zoster virus (VZV), a *Herpesviridae*, is the causative agent of the disease that shows little genetic variation (only one serotype is known). Following primary infection, the virus remains latent in neural ganglia, and upon subsequent reactivation VZV may cause zoster (shingles), a disease mainly affecting the elderly and immunocompromised persons, that consists in painful, circumscribed eruption of vesicular lesions with inflammation of associated dorsal root or cranial nerve sensory ganglia. IgM antibodies to varicella-zoster virus may be detected during primary and reactivated infection. Evaluation of specific immune status for VZV may guide management of immunosuppressed patients and administration of antiviral agents.

Varicella infection occurring in susceptible pregnant women can lead to severe, and even fatal, disease of the newborns. Infection occurring in the first four months of pregnancy can lead to child deformity, while infection at the time of delivery may cause life-threatening infection in newborns. Although individual cases may be prevented or modified by VZV immunoglobulin administration or treated with antiviral drugs, varicella can be controlled only by vaccination.

Analyzer

DiaSorin LIAISON®, DiaSorin, Inc. Stillwater, MN
Sunquest Method Code: **LIAS**

Sunquest Test Code

VZG: Varicella-zoster virus, IgG

Sample

Serum is the only acceptable specimen for this assay collected aseptically by venipuncture. Refer to specimen collection procedures.
Grossly hemolyzed, lipemic or particulate samples are not recommended

Minimum volume: 250 µL, actual test sample volume, 20 µL

Stability: 2-8 °C / 7 days, 45 days at -20 ºC or colder
Do not store in self-defrosting freezer.
Avoid repeated freeze thaw cycles, however samples demonstrate no significant difference with up to five freeze-thaw cycles.
Mix thawed samples prior to testing

Rejection criteria: Unlabeled tube

Preparation:

1. Whole blood specimens should be centrifuged following complete clot formation, according to Specimen Processing procedures.
2. Serum or plasma should be physically separated from cells as soon as possible with a maximum limit of two hours from the time of collection.
3. Lipemic samples should be ultrafuged.
4. Specimens should be free of particulate matter.
5. Transfer serum/ plasma or prepared urine to a properly labeled aliquot tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.

Reagents

LIAISON® VZV IgG (310495) Integral, (100 tests) supplied ready to use

Magnetic particles (2.5 mL)	[SORB]	Magnetic particles coated with varicella-zoster virus antigen (partially purified extract of infected cell cultures, ROD strain), BSA, PBS buffer, methylthiazolone and bromonitro-dioxane.
Calibrator 1 (2.7 mL)	[CAL|1]	Human serum or defibrinated plasma containing low varicella-zoster virus IgG levels, BSA, phosphate buffer, ProClin® 300 as a preservative, and an inert yellow dye. The calibrator concentration (Index) is referenced to the WHO 1st International Standard Preparation, 1987 (W1044).
Calibrator 2 (2.7 mL)	[CAL|2]	Human serum or defibrinated plasma containing low varicella-zoster virus IgG levels, BSA, phosphate buffer, ProClin® 300 as a preservative, and an inert yellow dye. The calibrator concentration (Index) is referenced to the WHO 1st International Standard Preparation, 1987 (W1044).
Specimen diluent (28 mL)	[DIL|SPE]	BSA, phosphate buffer, ProClin® 300 as a preservative, and an inert yellow dye.
Conjugate (23 mL)	[CONJ]	Mouse monoclonal antibodies to human IgG conjugated to an isoluminol derivative, BSA, PBS buffer, ProClin® 300 and gentamicin sulphate as preservatives.

Reagent Integral Storage and Stability:

- Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles.
- Stored sealed, the reagents are stable at 2-8°C up to the expiration date.
- After removing the seals, the Reagent Integral is stable for eight weeks when stored at 2-8°C or on board the LIAISON® Analyzer. Record new expiration date on the integral.
- Do not freeze.
- The Reagent Integral must not be used past the expiration date indicated on the kit and reagent integral labels.

Risk and Safety

- Reagents in this kit are classified in accordance with the US OSHA Hazard Communication Standard; for additional information see Safety Data Sheet available on www.diasorin.com
    - **Reagents:** Cal 1, Cal 2, Dil/Spe, may cause an allergic skin reaction.
        - Avoid breathing dust/fume/gas/mist/vapors/spray
        - Wear protective gloves/protective clothing/eye protection/face protection
        - Wash contaminated clothing before reuse
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents.
- Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands at the end of each assay
- Specimens should be handled at the BSL 2 level recommended for any potentially infectious human serum or blood specimen
- Do not eat, drink, smoke, or apply cosmetics in the assay laboratory
- Do not pipette by mouth
- Some reagents contain sodium azide as a preservative. Flush drains thoroughly with water after disposal
- Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious
- Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an *overkill* approach.

Reagent Integral Preparation

1.	Magnetic particles must be completely resuspended before the Integral is placed on the instrument.
2.	Before the seal is removed, rotate the small wheel at 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).
3.	Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended
4.	Remove the seal from each container of the Reagent Integral and turn the thumb wheel at the bottom of the magnetic particle container back and forth until the suspension turns brown.
5.	Carefully wipe the surface of each septum to remove residual liquid
6.	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.
7.	Place the Reagent Integral into the reagent area of the Analyzer with the bar code label facing left and let it stand for 30 minutes before use. The Analyzer automatically stirs and re-suspends the magnetic particles.
8.	Repeat as necessary until the magnetic particles are completely resuspended.
9.	Follow the Analyzer Operator's Manual to load the specimens and start the run.

Calibration

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows four calibrations to be performed. Refer to the Operator's Manual or LIAISON® Quick Guide for calibration instructions.
Recalibration in triplicate is required:

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- Every 8 weeks
- After servicing the LIAISON® Analyzer.
- If quality controls are out of acceptable ranges

Verify *new reagent lots* before use by testing Liaison VZV IgG controls, and a weakly positive patient sample (if available). Maintain a rack of previously tested samples for this purpose in the freezer. Document results and expected values on the calibration printout.

Comparable results verify the new reagent lot. Discrepant results must be resolved before the reagent can be used for patient testing.

Analytical Measuring Range (AMR)

VZV IgG is an FDA-cleared/approved in vitro diagnostic assay that reports the qualitative result based on a predefined cut-off value. Verification of AMR or the cut-off value is not required by CAP or CLIA.

Quality Control (Daily)

LIAISON® VZV IgG Controls (310496): intended for use as assayed quality control to monitor the performance of the DiaSorin Liaison VZV IgG assay on the Liaison Analyzer.

- Negative control (0.7 mL x 2 vials) human serum/plasma non-reactive for VZV IgG
- Positive control (0.7 mL x 2 vials) human serum/plasma reactive for VZV IgG
- Allow controls to reach room temperature prior to use.
- Return controls to the refrigerator immediately after each use.
- Control ranges should be entered manually in the analyzer software prior to loading a new lot of control vials on board.
- Controls are not kit lot specific and may be interchanged with different reagent kits

Frequency: Run 2 levels each day of use. Remove caps and load the bar-coded control vials into the “C” rack on the Liaison with the barcode facing out, or transfer 220 μL to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability:
Unopened: Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date
Opened: 8 weeks at 2-8°C between uses.

Sunquest Control Names:

Negative VZV = C-VZN
Positive VZV = C-VZP

Acceptable ranges: Refer to the certificate of analysis for ranges of control values established by DiaSorin in reliable assay runs. Acceptable ranges are maintained in the analyzer, and in Sunquest. Refer to the Quality Control Procedure for QC exception codes. Do not report patient results until control results are within expected ranges.

Procedure

Refer to the instrument Operating procedure.

- Strict adherence to the Operator's Manual ensures proper assay performance.
- Each test parameter is identified by the bar codes on the Reagent Integral label.
- In case of malfunction of the bar code reader, the cartridge cannot be read, and the integral cannot be used.

The Analyzer operations are as follows:

1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense coated magnetic particles.
3. Dispense specimen diluent.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Kit and measure the light emitted.

Interferences

The DiaSorin LIAISON® VZV IgG assay was evaluated for interference according to CLSI Document EP7 (5). None of the interferents at the levels tested below produced a change in clinical interpretation or a significant change of the assay.

- Hemolysis 1000 mg/dL
- Lipemia 3000 mg/dL
- Icterus 20 mg/dL

Reference Range

The prevalence of VZV antibodies can vary depending on age, geographical location, socioeconomic status, race, and vaccine usage. The prevalence of VZV antibodies generally varies from about 15% positive in two-year olds to about 95% in persons over 40 years of age.

Limitations

- Do not heat inactivate sera
- Test for VZV IgG antibodies is of use when clinical symptoms are present or infection suspected.
- Screening of the general population leads to no appreciable diagnostic advantage.
- Results from immunosuppressed patients should be interpreted with caution.
- The performance characteristics with individuals vaccinated with VZV (ROD Strain) have not been established.
- The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
- Diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgment.
- Although patient samples are washed away prior to the addition of the mouse monoclonal antibody (conjugate), the performance characteristics of HAMA and rheumatoid factor samples have not been established and may occasionally influence results.

Dilutions

Do not dilute.

Result Reporting

Review, validate, and tag results and send to Sunquest.
Release results in Sunquest following LIS procedures for OEM. Result Comments are automatically appended when resulting in OEM or MEM using the LIAS worksheet.

Index Value	Result	Result Comment	Interpretation
Below 135	Negative	Absence of detectable VZV IgG antibodies. If exposure to varicella-zoster virus is suspected despite a negative finding, a second sample should be collected and tested no less than one to two weeks later	Absence of detectable VZV IgG antibodies. A negative result indicates no detectable VZV antibody, but does not rule out acute infection. It should be noted that the test usually scores negative in infected patients during the incubation period and the early stages of infection.
135 - 165	Equivocal	A second sample should be collected and tested no less than one to two weeks later.	The equivocal sample should be repeat tested. In case the result remains in this range after repeat testing, a second sample should be collected.
Above 165	Positive	Presence of detectable VZV IgG antibodies. A positive result indicates current or past exposure to varicella-zoster virus	Presence of detectable VZV IgG antibodies. A positive result generally indicates exposure to the pathogen or administration of specific immunoglobulins, but it is no indication of active infection or stage of disease.

The Analyzer automatically calculates VZV IgG antibody concentrations expressed as U/mL and grades the results.

Warning - If the sample result displays "Invalid RLU" and an exclamation mark (!) flag, the result obtained lies below the assay signal range. The sample must be retested. If the sample result upon retest still displays "Invalid RLU", call DiaSorin Technical Support.

Note: Repeat all Negative Results, especially on employees, to confirm the negative response.

Note - *The magnitude of the measured result*, *above the cutoff, is not indicative of the amount of antibody present*.

Alternate Methods

- When test performance does not meet quality standards, consult the technical specialist or Medical Director, and refer testing to Mayo Medical Laboratory.
- Order test 8812, Varicella-Zoster Antibody, IgG, Serum, and submit 0.5 mL of serum.

Specimen Storage

Promptly stopper tested specimen and store upright in specimen rack. Store completed samples at <-20° C a minimum of 7 days in specimen storage freezer.

References

1. **LIAISON® VZV IgG PN 310495** Directions for Use, 6/2015, DiaSorin, Inc, Stillwater, MN 55082, 01/06/2015
2. **LIAISON® Control VZV IgG PN 310496** Directions for Use, DiaSorin, Inc, Stillwater, MN 55082, 01/06/2015

Appendices

Refer to LIAISON® VZV IgG (310495) Directions for Use for specific performance characteristics

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Marisa Gremillion/L. Lichty	August 15, 2011	Initial Version
2.	Linda Lichty	August 22, 2011	Added statements for clarification of reporting and QC handling.
3.	Linda Lichty	April 22, 2013	Update product insert
4.	Linda Lichty	August 26, 2013	Retest negative samples
5.	Linda Lichty	September 28, 2015	Revised reagent, eliminated Biorad control, extend calibration stability