VZV IgG
Enzyme Immunoassay

For Individual Laboratory to Complete:

Laboratory Name		
Adopted		
Reviewed		
Reviewed		
Revised		
Supercedes		

Method: **Diamedix Corp., Immunosimplicity®**
Manual or in conjunction with one of the Diamedix Automated EIA Systems such as the MAGO Plus, the DSX, or the DS2. For *In Vitro* Diagnostic Use.

Clinical Significance

Varicella (chickenpox) and zoster (shingles) represent different clinical manifestations of infection with the same agent, Varicella-Zoster Virus (VZV), a member of the *Herpesviridae*. Varicella occurs most frequently in children and is characterized by a generalized vesicular exanthem often accompanied by fever. Zoster usually occurs in adults or immunocompromised patients (including those with AIDS) and consists of a painful, circumscribed eruption of vesicular lesions with accompanying inflammation of associated dorsal root or cranial nerve sensory ganglia. Varicella is the primary infection with VZV,whereas zoster is a secondary infection due to reactivation of latent VZV in sensory ganglia. That zoster results from reactivation of latent virus rather than reintroduction of virus into the host is supported by the fact that zoster does not exhibit the seasonal prevalence seen with varicella (late winter and spring), nor does zoster frequently occur in young patients who are often exposed to their own children with chickenpox. Studies indicate that reinfection and reactivation of VZV may occur in the absence of clinical symptoms (1,2,3,4).

There are several situations in which providing a specific laboratory diagnosis for VZV infection is crucial. VZV infection may cause severe or fatal disease in individuals who are receiving immunosuppressive therapy or who have abnormalities in their cell-mediated immune responses. Progressive, generalized varicella occurs in as many as 30% of children who acquire chickenpox while receiving chemotherapy and radiotherapy for cancer, and mortality in these cases has ranged from 7 to 28%. In immunodeficient patients who have had varicella,there is an increased risk of disseminated zoster. Providing a specific diagnosis of VZV infection in immunosuppressed patients or their contacts may guide the administration of anti-viral agents. Determining the immune status in high-risk immunocompromised individuals and adults exposed to VZV infection also guides the management of these individuals. Varicella infections occurring

in susceptible pregnant women at the time of delivery may cause life-threatening infection in the newborn. An attenuated live VZV vaccine has been licensed in the U.S. for use in non-immunocompromised individuals (1,4).

The traditional methods of antibody detection such as complement fixation (CF), neutralization and immunofluorescence (IF) have been replaced by enzyme immunoassay (EIA) which is more sensitive, equally specific and less labor intensive (5,6,7,8).

The Diamedix® Immunosimplicity® VZV IgG Test Kit is an EIA procedure intended for the semi-quantitative and qualitative detection of antibodies to VZV antigen. The test can be performed either manually or in conjunction with one of the Diamedix Automated EIA Systems. The results are objective and reported in ELISA units (EU/ml), standardized against in-house reference materials.

Principle of the Procedure

Diluted samples are incubated with VZV antigen bound to the solid surface of a microtiter well. If IgG antibodies against VZV are present in the samples, they will bind to the antigen forming antigen-antibody complexes. Residual sample is eliminated by aspirating and washing. Conjugate (horseradish peroxidase-labeled anti-human IgG) is added and will bind to these complexes. Unbound conjugate is removed by aspiration and washing. Substrate is then added and incubated. In the presence of bound enzyme the substrate is converted to an end product. The absorbance of this end product can be read spectrophotometrically at 450 nm (reference 600-630 nm) and is directly proportional to the concentration of IgG antibodies to VZV antigen present in the sample.

Specimen Collection

Whole blood should be collected by accepted medical techniques. Separated serum should remain at 22°C for no longer than 8 hours. If assays are not completed within 8 hours, serum should be refrigerated (2 to 8°C). If assays are not completed within 48 hours, or the separated sample is to be stored beyond 48 hours, samples are to be frozen at -20°C. Prior to testing, bring frozen sera to room temperature slowly and mix gently, avoiding foam formation. Specimens containing visible particulate matter should be clarified by centrifugation before testing. Grossly contaminated, hemolyzed, lipemic, or icteric specimens should not be used.

For the diagnosis of acute VZV infection, the acute-phase specimen should be drawn as soon after onset as possible, preferably within the first 7 days. The convalescent-phase specimen should be drawn 10 or more days after the acute-phase specimen.

CAUTION: Serum samples must not be heat-inactivated prior to use.

Reagents

Antigen Wells	Twelve, 8-well microwell breakapart strips, color-coded gold, coated with Varicella-Zoster Virus antigen (partially purified extract of human fibroblasts infected with VZV), strain ELLEN (ATCC).
Calibrator	One vial with blue cap containing 1.8 ml of pre-diluted human serum, highly reactive for VZV IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
Positive Control	One vial with white cap containing 1.8 ml of pre-diluted human serum, moderately reactive for VZV IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Assigned EU/ml value printed on label.
Negative Control	One vial with black cap containing 1.8 ml of pre-diluted human serum, non-reactive for VZV IgG antibodies, 0.2% sodium azide and Proclin™ 300, 90ppm active ingredient. Note that Calibrator and controls are produced from different serum lots.
Sample A Diluent	One bottle with blue cap containing 60 ml Phosphate buffer with protein stabilizers. Contains 0.2% sodium azide and Proclin™ 300, 90 ppm active ingredient. Color-coded blue.
Wash S Concentrate	Two bottles with clear caps containing 50 ml of Phosphate buffered saline with Proclin™ 300, 15 ppm active ingredient. Color-coded light blue/green. Each bottle is sufficient to make 1 liter of wash solution.
Conjugate	One bottle with red cap containing 25 ml goat anti-human immunoglobulin G labeled with horseradish peroxidase. Also includes protein stabilizers and Proclin™ 300, 30 ppm active ingredient. Color-coded pink.
Substrate HRP	One amber bottle with brown cap containing 25 ml buffered TMB solution (3,3',5,5' tetramethylbenzidine).
Stop M Solution	One bottle with white cap containing 30 ml of 1 N Phosphoric and 1N Hydrochloric acids. **CAUTION:** Acids are corrosive. Avoid contact with skin or eyes. If contact is made, flush area with copious amounts of water. See Precautions section.

These reagents should be stored at 2 to 8° C.

Other Materials Required

Manual Users:

1. Wash bottle or automated microplate washer
2. Pipettors capable of dispensing appropriate volumes
3. Timer
4. One liter graduated cylinder
5. One liter wash solution reservoir
6. Deionized or distilled water
7. Absorbent toweling
8. Tubes or microwell plate for serum dilution
9. Reader capable of reading absorbance at 450nm, reference at 600 or 630 nm.

Diamedix Automated EIA Systems Users:

1. One liter graduated container
2. Deionized or distilled water
3. Dilution containers as appropriate to system
4. Sample and Reagent tips required by system
5. Reagent containers required by system

Warnings:

1. Handle samples, Calibrator, controls and the materials that contact them as potential biohazards. Each donor unit in the Calibrator and controls has been found negative for Hepatitis B surface antigen and HIV-I antibodies by FDA-approved third generation tests. However, because no method can offer complete assurance that HIV-1, Hepatitis B virus, or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual, "Biosafety in Microbiological and Biomedical Laboratories", 1988.
2. Never pipette by mouth.
3. Avoid contact with open skin and mucous membranes.
4. Certain of the test reagents contain Proclin™ 300 as a preservative. When disposing of reagents containing Proclin™ 300, flush drains with copious amounts of water to dilute the active components below active levels.
5. Serum components contain sodium azide as preservative. Azides are reported to react with lead and copper in plumbing to form compounds that may become explosive. When disposing of solutions containing sodium azide, flush with copious amounts of water to minimize the build up of metal azide compounds.
6. Sodium azide inhibits conjugate activity. Clean pipet tips MUST be used for conjugate addition so that azide is not carried over from other reagents.

7. Avoid contamination of the TMB substrate solution with conjugate or other oxidants which will cause the solution to change color prematurely.
8. The substrate contains 3,3' 5,5' Tetramethylbenzidine (TMB) which has been shown to cause possible mutagenic effects in laboratory experiments.

Calibration

This test uses an in-house reference standard (or Calibrator). This Calibrator has been prepared from a pool of sera strongly positive for the antibody under investigation. The Calibrator functions as an internal reference preparation and is assigned a unitage in ELISA units (EU) per ml. The Calibrator must be included in every test run.

These tests have been optimized to permit the use of single point calibration. This is possible because the dose response curves are sufficiently linear and pass near to, or through the origin. The linearity of the dose response has been validated by the manufacturer during quality control testing.

Patient samples which contain very high levels of antibody may produce absorbance values greater than the Calibrator absorbance. Patient sample results greater than the Calibrator value should be reported as "Greater than Calibrator value EU/ml". If numerical results are required for such samples, dilute the sample using Sample Diluent and re-assay. Several dilutions (for example 1/5, 1/10 and 1/20) of the pre-diluted sample may be re-assayed simultaneously. Select the dilution that has an absorbance reading about 50% of the absorbance reading of the Calibrator; calculate the EU/ml for this dilution and multiply by the dilution factor to obtain estimated values.

Quality Control

a) The Positive and Negative Controls must be included in each test run.
b) The absorbance of the Blank must be < 0.2.
c) The Positive Control must be within its assigned range.
d) The Negative Control must be < 15.0 EU/ml.

If any of these criteria is not met, the run is invalid and must be repeated.

Notes: The Negative and Positive Controls are intended to monitor substantial reagent failure. The controls will not control all parts of the procedure such as technical dilution of patient specimens. The Positive Controls will not ensure precision at the assay cut-off. Users may wish to establish an in-house control having a quantitative value determined by replicate testing, at or near the cut-off to monitor the precision of the assay cut-off. Additional controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
If paired sera controls are desired, it is recommended that a four-fold dilution of a positive sample of Calibrator strength, is made first in Sample Diluent and then diluted according to assay procedures.The undilute

and four-fold diluted material will provide a simulated serum pair. The four-fold dilution ratio is compared against the established ratio.

Procedure

Allow all test components and patient samples to warm to room temperature before use. Invert reagent bottles gently several times before use. Return promptly to the refrigerator after use.

Prepare Wash Solution by adding 50 ml of Wash Concentrate(20X) to one liter of deionized or distilled H_2O.

Manual Users:
The Calibrator and Controls are provided ready to use: DO NOT DILUTE FURTHER.

1. Prepare 1:101 dilutions of the patient samples in Sample Diluent. (e.g., by addition of 2 µl sample to 200 µl Sample Diluent or 5 µl sample to 500 µl Sample Diluent)
2. Mix sample dilutions gently by withdrawing and expelling in a pipette tip 2 or 3 times or by vortex mixing for 2 or 3 seconds. Transfer 100 µl of Calibrator, controls and diluted patient samples to the antigen wells. Avoid formation of bubbles when transferring diluted samples.

   NOTE: *Include one well which contains 100 µl of Sample Diluent only as the reagent blank. This will ultimately be used to "zero" the photometer before reading the test results.*
3. Allow the wells to incubate at room temperature(18-30°C) for 30 ± 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling if necessary. Wash the wells by rinsing 3 times with at least 300 µl per well of Wash Solution. Remove excess moisture from the wells after washing. When using an automated washer, follow the manufacturer's instructions.
5. Place 100 µl of Conjugate into each well, avoiding bubble formation.
6. Allow the wells to incubate uncovered at room temperature (18-30°C) for 30 ± 5 minutes.
7. Wash the wells as described in Step 4 above.
8. Place 100 µl of Substrate into each well, avoiding bubble formation.
9. Allow the wells to incubate uncovered at room temperature(18-30°C) for 30 ± 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of each well at 450 nm and zero against the reagent blank. A suitable reference wavelength (e.g.,600-630 nm) reading should be used. Read the plate within 60 minutes of adding Stop Solution.

Refer to the BP-96 Plate Reader Operation Manual for complete instructions on set-up and operating procedures.

Diamedix Automated EIA System Users:
If using one of Diamedix's Automated EIA Systems, please refer to the corresponding Operating Manual(s) for the test setup, procedure, and accessories/consumables needed.

Calculation of Results

Determine the EU/ml (ELISA Units/ml) for each patient specimen or control using the following formula:

$$\frac{\text{EU/ml of Calibrator}}{\text{Absorbance of Calibrator}} \times \text{Absorbance of Sample} = \text{EU/ml of sample}$$

Reference Ranges

The following is only a guide to interpretation. **Each laboratory can establish its own "normal" ranges based on populations encountered.**

Less than 15.0 EU/ml Index < 0. 75	Nonreactive (Negative) for anti-VZV IgG; presumed non-immune to VZV.
Greater than/equal to 20.0 EU/ml Index $\geq$ 1	Reactive (positive) for anti-VZV IgG; presumed immune to VZV.
15.0 to 19.9 EU/ml Index 0.75-0.99	Equivocal*

* When equivocal results are obtained, another specimen should be collected ten to fourteen days later and tested in parallel with the initial specimen. If the second sample is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample shows a significant increase in antibody level, the patient may be considered to have a primary infection. The conversion of an individual patient's serum from negative to positive for antibodies to the infectious agent in question, is defined as seroconversion, and indicates active or recent infection.

Note: The concentration of anti-VZV IgG in a given specimen determined from assays from different manufacturers can vary due to differences in assay methods and reagents.

Reporting Results

When the EU/ml value is reported for a single specimen the following statement should be included "The following results were obtained with the Diamedix Immunosimplicity Is-VZV IgG EIA Test System. The magnitude of the measured result, above the cut-off, is not indicative of the total amount of antibody present. The magnitude of the reported IgG level cannot be correlated to an endpoint titer".

When the assay is used semi-quantitatively, the following statement should be included when reporting results: "Timing of specimen collection for paired sera may be critical. In some patients, antibody titers may rise to significant levels and fall again to lower or undetectable levels within a month. Other patients may not develop significant antibody levels. Culture results, serology and antigen detection methods should all be appropriately used along with clinical findings for diagnosis".

Paired Sera

To determine a significant difference between acute/convalescent serum pairs, both specimens should be run within the same assay. In addition, paired sera should be evaluated within the linear range of the assay. The upper limit of the linear range has been set at 100 EU/ml. In-house studies performed manually and using Diamedix Automated EIA Systems have shown that a 2.8-fold or greater increase in EU/ml ratio (convalescent serum EU/ml value / acute serum EU/ml value) corresponds to a four-fold incease in VZV IgG antibody level and a 1.8-fold increase in the EU/ml ratio corresponds to a two-fold incease in VZV IgG antibody level. Ratios in the range of 1.8 to 2.8 may be considered equivocal for significant increase status. In this case, paired samples can be retested or additional samples collected if necessary.

Procedure Notes

1. Do not interchange reagents from different reagent lots except for Sample **A** Diluent, Wash **S** Concentrate, Substrate **HRP** and Stop **M** Solution.
2. Do not use reagents beyond their expiration date.
3. Store unused reagents at 2 to 8 ^{o}C.
4. Incubations above or below the recommended temperatures or times may give erroneous results.
5. The EIA method is a very sensitive technique. Maintain consistent pipetting technique, incubation times, and temperature conditions throughout the test procedure. Cross contamination between reagents can invalidate the test.
6. Antigen coated microwells should be stored with the desiccant in the resealable bag provided and returned to the refrigerator immediately after use.
7. *(Manual Procedure Only)* The washing procedure is very important and requires special attention. (Please refer to the Procedure section)

   NOTE: *Improperly washed* wells *may* give *erroneous results.*

Limitations

1. The performance characteristics with individuals vaccinated with VZV(OKA Strain) have not been established.
2. The results obtained with the Is-VZV IgG Test Kit serve only as an aid to diagnosis and should not be interpreted as diagnostic in themselves.

3. Assay performance characteristics have not been established for visual result determination.
4. The Diamedix Immunosimplicity Is-VZV IgG Test System is linear from 20.0 EU/ml(1.0 Index Value) to 100 EU/ml.
5. The test should be performed on serum. The use of whole blood, cord blood or plasma has not been established.
6. A single positive result only indicates previous immunologic exposure; the level of antibody response or class of antibody may not be used to determine active infection or disease stage.
7. A negative result does not rule out the diagnosis of VZV infection. The sample may have been collected before appearance of detectable antibodies. Negative results in suspected early VZV infection should be repeated in 2-3 weeks.
8. Positive results from cord blood or neonates should be interpreted with caution.
9. Results from immunocompromised patients should be interpreted with caution.
10. Heterotypic antibody titer rises in response to VZV may occur in certain patients with HSV infection who have experienced a prior infection with VZV (9).
11. The performance characteristics of the Diamedix Is-VZV IgG Test Kit with automated equipment other than Diamedix Automated EIA Systems have not been established.

References

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