Diamedix®
A Subsidiary of ERBA Diagnostics, Inc.

**VZV IgG**
*Enzyme Immunoassay Test Kit*
**For *In Vitro* Diagnostic Use**
**Catalog No. 720-380 - 96 Tests**

## SUMMARY OF PROCEDURE

1. Prepare 1:101 dilutions of patient samples in Sample Diluent. Mix well. Note that Calibrator and Controls are ready-to-use.
2. Add 100 µl of Calibrator, Controls and diluted samples into the antigen wells. Reserve one well for reagent blank (100 µl of Sample Diluent).
3. Incubate at room temperature (18-30°C) for 30 ± 5 min.
4. Discard contents of the wells. Wash the wells 3 times with Wash Solution.
5. Add 100 µl of Conjugate to each well.
6. Incubate at room temperature (18-30°C) for 30 ± 5 min.
7. Wash the wells as in #4 above.
8. Add 100 µl Substrate Solution to each well.
9. Incubate at room temperature (18-30°C) for 30 ± 5 min.
10. Add 100 µl Stop Solution to each well.
11. Read the absorbances at 450/600-630 nm.

## INTENDED USE

For the qualitative and semi-quantitative detection of IgG antibodies to Varicella-Zoster Virus (VZV) in human serum by indirect enzyme immunoassay to determine a prior exposure to VZV and, when evaluating paired sera, to aid in the determination of acute or convalescent stage of VZV infection.

## SUMMARY AND EXPLANATION

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® (*Is*)-VZV IgG test kit is an EIA procedure intended for the semi-quantitative and qualitative detection of antibodies to VZV antigen. 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.

## REAGENTS

***Each Is-VZV IgG Test Kit contains reagents for 96 tests.***

| | |
|---|---|
| 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 | Two vials with blue caps 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 range 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 (20X) | 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 1050 ml 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 preservatives. 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. |

**Store these reagents at 2 to 8°C.**

## OTHER MATERIALS REQUIRED

**Manual Users:**

Wash bottle or automated microplate washer.
Pipettors capable of dispensing appropriate volumes.
Timer.
One liter graduated cylinder.
One liter wash solution reservoir.
Deionized or distilled water.
Absorbent toweling.
Tubes or microwell plate for sample dilution.
Reader capable of reading absorbance at 450nm, reference at 600-630 nm.

**Automated EIA Processor Users:**

One liter graduated cylinder.
Deionized or distilled water.
Pre-dilution cups, strips or plates.
ProbeClean™ Concentrate, or tip washing detergent solution, if applicable.

## WARNINGS AND PRECAUTIONS

REAGENTS: For *in vitro* Diagnostic Use.

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", 1993.
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 components below active levels.
5. Reagents containing Sodium Azide:

   (a) **CAUTION:** Some reagents in this kit contain Sodium Azide as preservative. Sodium Azide may react with lead or copper plumbing to form highly explosive metal azides. On disposal, flush with a large volume of water to prevent azide build-up. For further information, refer to "Decontamination of Laboratory Sink Drains to Remove Azide Salts", in the Manual Guide – Safety Management No. CDC-22, issued by the Centers for Disease Control and Prevention, Atlanta, GA, 1976.[11]

   **European Communities Hazardous Substance Risk Phrases (Regulation (EC) No 1272/2008)**

   H300 –Fatal if swallowed.
   H310 – Fatal if contact with skin.
   EUH032 – Contact with acids liberates very toxic gas.
   H410 – Very toxic to aquatic life with long lasting effect.
   P264 – Wash all exposed external body areas thoroughly after handling.
   P302+P352 – IF ON SKIN: Wash with plenty of water and soap.
   P301+P310/P330 – IF SWALLOWED: Immediately call a POISON CENTER or doctor/physician. Rinse mouth.
   P270 – Do not eat, drink or smoke when using this product.
   P501 – Dispose of contents/container as hazardous waste.
   P391 – Collect spillage.
   P273 – Avoid release to the environment. Refer to special instructions/ Safety Data Sheet.

   (b) Sodium Azide inhibits horseradish peroxidase activity. Care must be taken to ensure that azide is not carried over from other reagents into conjugate and substrate steps.
6. Avoid contamination of the TMB substrate solution with conjugate or other oxidants, which will cause the solution to change color prematurely.

ADDITIONAL PRECAUTIONS:

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. Expiration dates are printed on the reagent labels.
3. Store unused reagents at 2 to 8°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*.

## 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.*

## 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 ul of Sample Diluent 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 to 30°C) for 30 $\pm$ 5 minutes.
4. Aspirate or discard the contents of the wells. Remove any excess moisture in the wells by tapping on paper toweling. Wash the wells by rinsing 3 times with at least 300 µl 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 to 30°C) for 30 $\pm$ 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 to 30°C) for 30 $\pm$ 5 minutes.
10. Place 100 µl of Stop Solution into each well, avoiding bubble formation.
11. Read the absorbance of the wells at 450 nm using a reference wavelength of 600-630 nm. The plate should be read within 60 minutes of adding Stop Solution.

### AUTOMATED EIA PROCESSOR USERS:

When using an Automated EIA Processor, refer to the Operator's Manual for the test setup and procedures.

***NOTE:*** *Automated EIA Processor users must validate their equipment to demonstrate that the results obtained are equivalent to those obtained using manual assay.*

## QUALITY CONTROL

1. The Positive and Negative Controls must be included in each test run.
2. The absorbance of the Blank must be < 0.2.
3. The Positive Control must be within its assigned range.
4. The Negative Control must be < 15.0 EU/ml.

If any of these criteria are not met, the results are invalid and the test should 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 Control 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 undiluted and 4-fold diluted material will provide a simulated serum pair. The four-fold dilution ratio is compared against the established ratio.

## RESULTS

### Single Point Calibration

The Diamedix *Is*-VZV IgG Test Kit has been developed using a single point calibrator. The use of a single point calibrator is possible since the test system has been shown to be linear from the cut-off up to the Calibrator value (100 EU/ml). This linear response has been validated both manually and using MAGO Plus by testing serial dilutions of the Calibrator. $R^2$ values obtained for both manual and MAGO Plus were greater than 0.98. If users wish to check the linear response they may do so by testing serial two-fold dilutions of the Calibrator.

### 1. Calculation

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

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

Patient values, which produce absorbance values above the Calibrator absorbance, may be reported as "greater than 100 EU/ml". Alternatively such samples may be pre-diluted using Sample Diluent and re-assayed. 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.

Index values can be calculated by dividing the EU/ml values obtained by 20 (the positive cut-off value).

An Automated EIA Processor (e.g. MAGO® Plus Automated EIA Processor) will calculate and print results using the above formula.

### 2. Interpretation of Results

| EU/ml | Index Value | Interpretation |
|---|---|---|
| < 15.0 EU/ml | 0.75 | Nonreactive (Negative) for anti-VZV IgG: presumed non-immune to VZV |
| 15.0-19.9 EU/ml | 0.75-0.99 | Equivocal* |
| ≥ 20.0 EU/ml | ≥ 1.0 | Reactive (Positive) for anti-VZV IgG: presumed immune to VZV |

* 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.

### 3. 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."

### 4. 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 the MAGO Plus 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 increase in VZV IgG antibody level and a 1.8-fold increase in the EU/ml ratio corresponds to a two-fold increase 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.

## CUT-OFF ESTABLISHMENT

The Diamedix *Is*-VZV IgG cut-off was established to optimally differentiate those individuals with, from those without, immunological experience to VZV. The optimal cut-off value was determined by statistical analysis of the results of 108 sera shown to be negative in the *Is*-VZV IgG Test Kit as well as other test methods. The mean plus 3 SD (4.65 plus 3 x 3.67 = 15.66, rounded to 15.0) was considered the equivocal decision point. The mean plus 4 SD (4.65 plus 4 x 3.67 = 19.33, rounded to 20.0) was considered the positive cut-off point.

This cut-off value was further verified by applying the principles from Receiver-Operating Characteristic (ROC) Curves to 652 sera tested manually in the *Is*-VZV IgG test Kit and another commercially available test method. At the optimized cut-off level, the Diamedix *Is*-VZV IgG Test Kit has a relative sensitivity of 96% and a relative specificity of 98% based on comparison to the marketed test.

## 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 *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 the MAGO Plus Automated EIA Processor have not been established.

## EXPECTED VALUES

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 2 year olds to about 95% in persons over 40 years of age. Sera from 100 healthy South Florida blood donors (52 female and 48 male) were evaluated in the *Is*-VZV IgG Test Kit. Of the 100 samples, 92 were found to be reactive (positive), 6 were found to be non-reactive (negative) and 2 sera were equivocal. Age distribution, geographic location and prevalence is provided in Table 1. Histograms demonstrating the distribution of EU/ml values are shown in Figures 1 and 2.

**TABLE 1**

| | Number of donors | Prevalence |
|---|---|---|
| Total Number | 100 | 92.0% |
| Geographic Location: | | |
| South Eastern US | 100 | 92.0% |
| Age | | |
| 10-19 | 13 | 92.3% |
| 20-29 | 23 | 91.3% |
| 30-39 | 40 | 87.5% |
| 40-49 | 13 | 100.0% |
| 50-59 | 5 | 100.0% |
| 60-69 | 6 | 100.0% |

**FIGURE 1**
***Is* VZV IgG Reactive (Positive) Population**

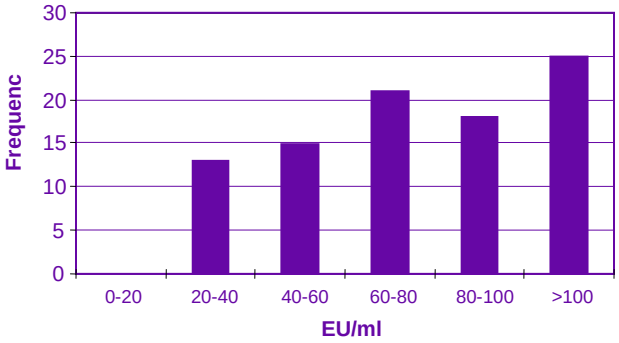
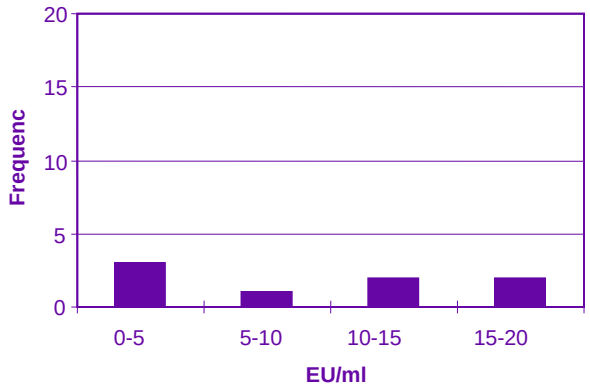



**FIGURE 2**
***Is* VZV IgG Non-Reactive (Negative) Population**




## PERFORMANCE CHARACTERISTICS

### A. Comparison Testing

A total of six hundred and fifty-two sera were tested for the presence of VZV IgG antibodies using the Diamedix *Is*-VZV IgG Test Kit and another commercially available EIA test kit at two independent sites (site #1, Miami, FL and site #2, Salt Lake City, Utah) as well as at Diamedix Corp., Miami FL (site #3). At site #3, testing was performed both manually and using the MAGO Plus Automated EIA processor. Site #1 tested 200 sera (all frozen). Samples were obtained from the S. Florida area. Thirty-seven of the samples were obtained from males, including 5 children, and 97 from females. Of the 97 samples from females, 46 (47%) were of child-bearing age (18-45 years). No age or gender data was available on the remaining samples. Table 2 compares the results obtained for the *Is*-VZV IgG Test Kit and their currently used testing method.

Site #2 tested 198 sera (all fresh). Samples were obtained from the Mid-West/West region. Fifty-eight of the samples were obtained from males, including 6 children, and 127 from females, including 1 child. Of the 127 samples from females, 107 (84%) were of child-bearing age (18-45 years). No age or gender data was available on the remaining samples. Table 3 compares the results obtained for the *Is*-VZV IgG Test Kit and their currently used testing method.

**TABLE 2**
***Is* VZV IgG – Site #1**

| | | Positive | Negative | Equivocal |
|---|---|---|---|---|
| Other EIA | Positive | 171 | 1 | 3 |
| | Negative | 0 | 23 | 2 |
| | Equivocal* | 0 | 0 | 0 |

| | | *95%CI*** |
|---|---|---|
| Relative Sensitivity | 171/172 = 99.4% | 96.8-100.0 |
| Relative Specificity | 23/23 = 100.0% | 85.2-100.0 |
| Overall Agreement | 194/195 = 99.5% | 97.2-100.0 |

**TABLE 3**
***Is* VZV IgG – Site #2**

| | | Positive | Negative | Equivocal |
|---|---|---|---|---|
| Other EIA | Positive | 164 | 4 | 6 |
| | Negative | 0 | 19 | 2 |
| | Equivocal* | 0 | 3 | 0 |

| | | *95%CI*** |
|---|---|---|
| Relative Sensitivity | 164/168 = 97.6% | 94.0-99.3 |
| Relative Specificity | 19/19 = 100.0% | 82.4-100.0 |
| Overall agreement | 183/187 = 97.9% | 94.6-99.4 |

*Equivocal results were excluded from calculations.
** Calculated by the Exact Method (10).

For site #1, the discordant sample was equivocal when tested by a referee EIA method. For site #2, the discordant sera were not available for further resolution.

Site #3 (Diamedix Corp.) tested 254 samples (all frozen) by the manual method and 253 of these samples (one being QNS) by the MAGO Plus method. No age or gender data was available for these samples. Of the samples tested 74 were specifically selected either because they were negative or had values close to the cut-off by other EIA methods. The remainder of the samples were obtained from the normal S. Florida blood donor population. Tables 4 and 5 compare the results obtained by manual and MAGO Plus testing for the *Is*-VZV IgG Test Kit and another marketed EIA method.

**TABLE 4**
***Is* VZV IgG – Site #3 (Manual)**

| | | Positive | Negative | Equivocal |
|---|---|---|---|---|
| Other EIA | Positive | 169 | 3 | 6 |
| | Negative | 2 | 68 | 6 |
| | Equivocal* | 0 | 0 | 0 |

| | | *95%CI*** |
|---|---|---|
| Relative Sensitivity | 169/172 = 98.3% | 95.0-99.6 |
| Relative Specificity | 68/70 = 97.1% | 90.1-99.7 |
| Overall Agreement | 237/242 = 97.9% | 95.2-99.3 |

**TABLE 5**
***Is* VZV IgG – Site #3 (MAGO Plus)**

| | | Positive | Negative | Equivocal |
|---|---|---|---|---|
| Other EIA | Positive | 173 | 1 | 3 |
| | Negative | 5 | 67 | 4 |
| | Equivocal* | 0 | 0 | 0 |

| | | *95%CI*** |
|---|---|---|
| Relative Sensitivity | 173/174 = 99.4% | 96.8-100.0 |
| Relative Specificity | 67/72 = 93.1% | 84.5-97.7 |
| Overall Agreement | 240/246 = 97.6% | 94.8-99.1 |

*Equivocal results were excluded from calculations.
** Calculated by the Exact Method (10).

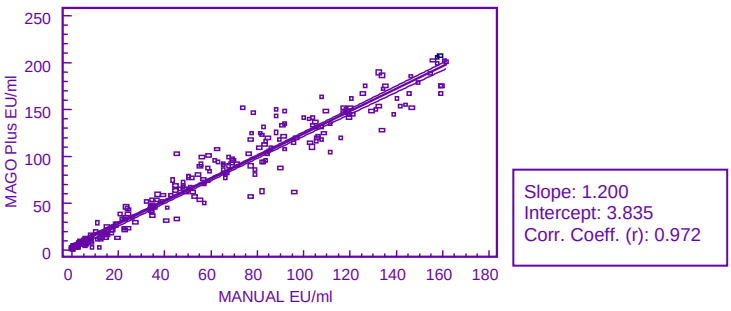
For site #3 (manual testing), the two samples that were positive by the *Is*-VZV IgG and negative by the other EIA were negative by a referee EIA method. For the three samples that were negative in the *Is*-VZV IgG Test Kit and positive by the other EIA one was negative, one was equivocal and one was positive in the referee method. For MAGO Plus testing the five samples that were positive in the *Is*-VZV IgG and negative by the other EIA were negative in the referee EIA method. The sample that was negative in the *Is*-VZV IgG Test Kit and positive by the other EIA was negative in the referee EIA method.

NOTE: Please be advised that 'relative' refers to the comparison of the assay's results to that of a similar assay. There was not an attempt to correlate the assay's results with disease presence or absence. No judgment can be made on the comparison assay's accuracy to predict disease.

## B. Correlation of Manual and MAGO Plus Results

The Diamedix *Is*-VZV IgG Test System has been developed for use both manually and using the MAGO Plus Automated EIA Processor. To further demonstrate the equivalence of the manual and MAGO Plus procedures, the results of the 253 serum samples tested by both methods were plotted. A scattergram and regression line of the results obtained with 95% confidence intervals is shown in Figure 3 and demonstrates good correlation.

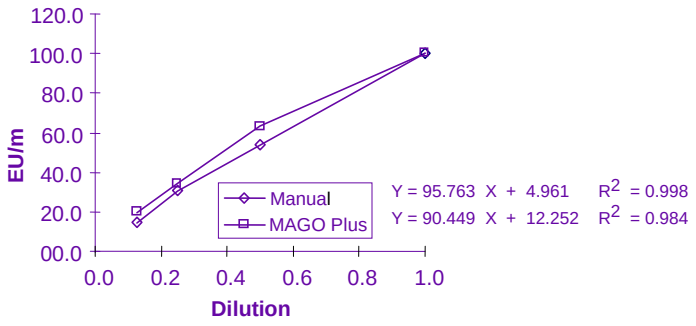
**FIGURE 3**




## C. Linearity

Several strongly positive serum specimens as well as the in-house standard were diluted (2-fold) and separate dilutions were assayed in the *Is*-VZV IgG Test Kit both manually and using the MAGO Plus Automated Processor. $R^2$ values for the samples ranged from 0.972 to 0.999 The titration curve of the in-house standard, manually and by MAGO Plus, is shown in Figure 4. The results demonstrate a high degree of linearity throughout the reportable range of the assay.

**FIGURE 4**




The linearity/dynamic range of the assay was determined to be 20-100 EU/ml.

## D. Semi-Quantitative Data

Serum pairs were obtained by preparing multiple serial 2-fold dilutions of several strongly positive sera. Ratios for dilutions representing a four-fold and two fold difference in antibody level were evaluated as a serum pair both manually and using the MAGO Plus. The overall mean ratio obtained for 4-fold dilutions was 3.13 (SD 0.35) and the overall mean ratio obtained for 2-fold dilutions was 1.77 (SD 0.15). Overall, it was estimated a ratio of 2.8-fold or greater (mean ratio minus 1 SD) increase in *Is*-VZV IgG EU/ml values corresponded to a four-fold increase in VZV IgG antibody levels. A ratio in the range of 1.8 to 2.8 was considered equivocal for significant increase determination.

## E. Cross Reactivity

Sera containing IgG antibodies to viruses potentially cross-reactive to VZV have been tested in the *Is*-VZV IgG Test Kit. Thirteen sera negative for antibodies to VZV in the *Is*-VZV IgG Test Kit as well as in another marketed test were positive for antibodies to one or more viruses. The data in the following table indicate that no cross-reactivity should be expected with the *Is*-VZV IgG Test Kit from these analytes.

**TABLE 6**

| Analyte | VZV IgG | HSV IgG | Measles IgG | CMV IgG | Rubella IgG |
|---|---|---|---|---|---|
| # of Pos. Samples | 0 | 12 | 12 | 9 | 12 |

## F. Precision

Six serum samples as well as the *Is*-VZV IgG Test Kit Calibrator, Positive Control and Negative Control, were assayed in triplicate in three separate runs for site #1 and site #2 and in six separate runs for site #3. The precision studies were performed manually at the two outside testing sites (site #1 and site #2) and at site #3 (Diamedix Corp.) both manually and using the MAGO Plus Automated EIA processor. The results obtained are shown in Tables 7-11.

**TABLE 7**
**Site #1 Intra- and Interassay Precision**

| SERUM | INTRA-ASSAY DAY 1 | | | INTRA-ASSAY DAY 2 | | | INTRA-ASSAY DAY 3 | | | INTERASSAY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% |
| A | 1.5 | 0.10 | 6.67 | 1.5 | 0.25 | 16.67 | 1.5 | 0.17 | 11.33 | 1.5 | 0.16 | 10.67 |
| B | 3.9 | 0.25 | 6.41 | 4.1 | 0.23 | 5.61 | 4.1 | 0.26 | 6.34 | 4.0 | 0.23 | 5.75 |
| C | 16.9 | 1.45 | 8.58 | 16.8 | 1.56 | 9.29 | 17.4 | 0.83 | 4.77 | 17.0 | 1.18 | 6.94 |
| D | 49.5 | 3.43 | 6.93 | 49.1 | 3.44 | 7.01 | 48.0 | 3.20 | 6.67 | 48.9 | 2.99 | 6.11 |
| E | 81.9 | 5.58 | 6.81 | 81.2 | 5.14 | 6.33 | 84.3 | 1.84 | 2.18 | 82.4 | 4.16 | 5.05 |
| F | 102.4 | 2.67 | 2.61 | 101.6 | 2.68 | 2.64 | 101.9 | 2.32 | 2.28 | 102.0 | 2.24 | 2.20 |
| CAL | 93.4 | 2.76 | 2.96 | 93.0 | 3.10 | 3.33 | 92.4 | 3.55 | 3.84 | 93.0 | 2.76 | 2.97 |
| POS | 44.3 | 1.10 | 2.48 | 44.1 | 1.18 | 2.68 | 42.1 | 0.91 | 2.16 | 43.5 | 1.38 | 3.17 |
| NEG | 1.3 | 0.06 | 4.62 | 1.3 | 0.00 | 0.00 | 1.1 | 0.15 | 13.64 | 1.2 | 0.15 | 12.50 |

**TABLE 8**
**Site #2 Intra- and Interassay Precision**

| SERUM | INTRA-ASSAY DAY 1 | | | INTRA-ASSAY DAY 2 | | | INTRA-ASSAY DAY 3 | | | INTERASSAY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% |
| A | 1.4 | 0.38 | 27.14 | 2.1 | 0.15 | 7.14 | 1.5 | 0.23 | 15.33 | 1.7 | 0.38 | 22.35 |
| B | 4.9 | 1.59 | 32.45 | 6.2 | 0.35 | 5.65 | 5.7 | 0.76 | 13.33 | 5.6 | 1.07 | 19.11 |
| C | 15.3 | 4.38 | 28.63 | 20.2 | 1.46 | 7.23 | 18.7 | 1.55 | 8.29 | 18.1 | 3.27 | 18.07 |
| D | 34.8 | 3.91 | 11.24 | 45.7 | 0.50 | 1.09 | 41.3 | 1.37 | 3.32 | 40.6 | 5.19 | 12.78 |
| E | 77.4 | 14.11 | 18.23 | 83.6 | 4.12 | 4.93 | 77.9 | 3.65 | 4.69 | 79.6 | 8.14 | 10.23 |
| F | 75.3 | 2.76 | 3.67 | 105.4 | 1.65 | 1.57 | 103.1 | 8.95 | 8.68 | 94.6 | 15.26 | 16.13 |
| CAL | 96.5 | 4.87 | 5.05 | 102.4 | 3.22 | 3.14 | 94.3 | 5.18 | 5.49 | 97.7 | 5.32 | 5.45 |
| POS | 45.8 | 2.41 | 5.26 | 55.2 | 3.35 | 6.07 | 51.0 | 2.75 | 5.39 | 50.7 | 4.78 | 9.43 |
| NEG | 1.6 | 0.03 | 18.75 | 1.8 | 0.51 | 28.33 | 2.0 | 0.76 | 38.00 | 1.82 | 0.52 | 28.89 |

**TABLE 9**
**Site #3 Intra- and Interassay Precision (Manual)**

| SERUM | INTRA-ASSAY DAY 1 | | | INTRA-ASSAY DAY 2 | | | INTRA-ASSAY DAY 3 | | | INTERASSAY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% |
| A | 1.7 | 0.08 | 4.90 | 1.9 | 0.10 | 5.26 | 1.8 | 0.10 | 5.56 | 1.8 | 0.14 | 7.78 |
| B | 4.4 | 0.45 | 10.17 | 4.7 | 0.53 | 11.26 | 4.3 | 0.35 | 8.06 | 4.4 | 0.37 | 8.41 |
| C | 18.2 | 0.81 | 4.43 | 17.7 | 1.03 | 5.79 | 17.1 | 0.58 | 3.37 | 17.5 | 0.89 | 5.09 |
| D | 46.7 | 1.64 | 3.51 | 46.3 | 1.47 | 3.19 | 45.3 | 1.15 | 2.54 | 45.5 | 2.63 | 5.78 |
| E | 71.7 | 2.78 | 3.88 | 71.6 | 4.12 | 5.76 | 68.3 | 1.70 | 2.49 | 70.3 | 3.09 | 4.40 |
| F | 99.3 | 5.12 | 5.16 | 99.9 | 4.03 | 4.03 | 95.2 | 6.53 | 6.86 | 97.9 | 5.38 | 5.50 |
| CAL | 97.8 | 7.15 | 7.31 | 98.6 | 7.35 | 7.46 | 98.3 | 5.26 | 5.36 | 97.1 | 6.25 | 6.44 |
| POS | 48.8 | 1.38 | 2.83 | 47.6 | 2.54 | 5.33 | 47.6 | 2.48 | 5.29 | 47.5 | 2.09 | 4.40 |
| NEG | 1.8 | 0.26 | 14.62 | 1.8 | 0.15 | 8.65 | 1.6 | 0.15 | 9.35 | 1.7 | 0.21 | 12.35 |

**TABLE 10**
**Site #3 Intra- and Interassay Precision (MAGO Plus)**

| SERUM | INTRA-ASSAY DAY 1 | | | INTRA-ASSAY DAY 2 | | | INTRA-ASSAY DAY 3 | | | INTERASSAY | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% | MEAN | SD | CV% |
| A | 2.7 | 1.07 | 40.23 | 2.4 | 0.71 | 29.95 | 3.0 | 0.29 | 9.48 | 2.7 | 0.77 | 28.52 |
| B | 4.4 | 0.77 | 17.58 | 4.6 | 0.92 | 19.95 | 5.4 | 0.50 | 9.14 | 4.8 | 0.84 | 17.50 |
| C | 19.9 | 1.97 | 9.92 | 21.1 | 1.11 | 5.27 | 21.4 | 1.31 | 6.13 | 20.8 | 1.56 | 7.50 |
| D | 51.3 | 1.75 | 3.41 | 52.2 | 3.60 | 6.90 | 58.1 | 4.02 | 6.91 | 53.9 | 4.39 | 8.14 |
| E | 91.2 | 10.07 | 11.04 | 96.3 | 10.26 | 10.65 | 98.5 | 15.09 | 15.32 | 95.3 | 11.73 | 12.31 |
| F | 108.0 | 6.47 | 5.99 | 117.1 | 6.48 | 5.53 | 117.7 | 8.03 | 6.82 | 114.3 | 8.03 | 7.03 |
| CAL | 112.8 | 10.45 | 9.26 | 119.0 | 8.80 | 7.39 | 128.5 | 13.17 | 10.25 | 120.1 | 12.23 | 10.18 |
| POS | 49.8 | 4.16 | 8.35 | 53.5 | 3.17 | 5.93 | 55.6 | 4.65 | 8.37 | 52.9 | 4.52 | 8.54 |
| NEG | 1.6 | 0.45 | 27.56 | 2.9 | 1.00 | 34.62 | 3.6 | 1.27 | 35.21 | 2.7 | 1.24 | 45.93 |

**TABLE 11**
**Inter-Site Precision (Manual)**

| SERUM | Site #1 | Site #2 | Site #3 | INTER-SITE | | |
|---|---|---|---|---|---|---|
| | MEAN EU/ml | MEAN EU/ml | MEAN EU/ml | MEAN EU/ml | SD | CV% |
| A | 1.5 | 1.7 | 1.8 | 1.7 | 0.15 | 8.82 |
| B | 4.0 | 5.6 | 4.4 | 4.7 | 0.83 | 17.66 |
| C | 17.0 | 18.1 | 17.5 | 17.5 | 0.55 | 3.14 |
| D | 48.9 | 40.6 | 45.0 | 45.0 | 4.17 | 9.27 |
| E | 82.4 | 79.6 | 70.3 | 77.4 | 6.33 | 8.18 |
| F | 102.0 | 94.6 | 97.9 | 98.2 | 3.71 | 3.78 |
| CAL | 93.0 | 97.7 | 97.1 | 95.9 | 2.56 | 2.67 |
| POS | 43.5 | 50.7 | 47.5 | 47.2 | 3.61 | 7.65 |
| NEG | 1.2 | 1.8 | 1.6 | 1.6 | 0.32 | 20.00 |

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