INSTRUCTIONS FOR USE

EN

Anti-VZV IgG

REF 9Z9331G
SM9Z9331G
9Z9331GB

IVD

CE

Rx Only

Σ 96

Σ 480

INTENDED USE

The Anti-VZV IgG is intended for the qualitative detection of VZV IgG antibody in human serum. When performed according to these instructions, the results of this test, together with other clinical information, may aid in the determination of immune status, and/or aid in the diagnosis of VZV infections. This test is intended for *In Vitro* diagnostic use.

SIGNIFICANCE AND BACKGROUND

Varicella-Zoster virus (VZV) is a common pathogen in humans. The clinical course of VZV in humans is generally categorized into varicella (chickenpox) and *Herpes zoster* (shingles). The major significant advance in understanding the nature of these agents was originally contributed by Weller and co-workers who demonstrated the method for isolation and serial propagation of the virus (1, 2), and more recently, the epidemiology and control (3). Viral isolates obtained from patients with chickenpox and zoster were demonstrated to be identical on the basis of cytopathic effect (1), antigenicity (2), and morphology (4, 5). More recently, these viruses have been shown to have identical DNA molecular weight (6), and restriction endonuclease patterns (7).

The clinical symptoms of primary varicella (chickenpox) include a prodromal period of headaches, malaise, and fever preceding the exanthem, or the characteristic eruptions may be the first symptom. The rash is pleomorphic and goes through evolution from macular to papular, and then to vesicular stage. The rash characteristically develops in successive crops of new lesions over a three to five day period.

Chickenpox is endemic in the United States and generally affects children in the primary school bracket (five to eight years). Adults, adolescents, and newborns are also susceptible to infection. The disease appears in two to five year cycles, usually in the winter or spring, and may reach epidemic levels. Varicella infections during early pregnancy rarely have been found to cause congenital anomalies. Varicella infections occurring in susceptible pregnant women at the time of delivery may have a life-threatening infection in the newborn, as well as patients in a variety of pathologies (8 - 10). The potential spread of a nosocomial disease is not uncommon.

Herpes zoster (shingles) is a disease primarily of adults, with most of the cases occurring in the age group over 50 years. In contrast to the epidemic and seasonal nature of varicella (chickenpox) infection, *Herpes zoster* has a random pattern of occurrence. *Herpes zoster* is believed to be the reactivation of a pre-existing varicella virus which has been in a latent state since the occurrence of primary varicella infection. Persons affected with *Herpes zoster* infections do so even in the presence of pre-existing antibody levels to varicella virus. Symptoms of *Herpes zoster* are erythematous, maculopapular areas which develop over an area of skin served by an afferent nerve. Single or clumps of vesicles then appear, usually accompanied by pain which, in some cases, can be extreme (11).

Based on the epidemiologic evidence that VZV is spread by droplet nuclei or air droplets, and possibly by skin squames, the portal of entry of the virus is assumed to be through the respiratory passages (12). After dissemination of VZV from the blood, it rapidly spreads to the skin and is detectable in the endothelium, and then involves the cells of the epidermis with accumulation of fluid between the prickle cell layer and outer epidermis forming a vesicle (13). The vesicle becomes the site of intense immunologic activity with initial infiltration of polymorphonuclear leukocytes that remain the predominant inflammatory cell as observed in *Herpes zoster* (14). Later, mononuclear cells migrate into the vesicle.

PRINCIPLE OF THE ASSAY

The Anti-VZV IgG is designed to detect IgG class antibodies to VZV in human sera. Creation of the sensitized wells of the plastic microwell strips occurred using passive absorption with VZV antigen. The test procedure involves three incubation steps:

1. Test sera (properly diluted) are incubated in antigen coated microwells. Any antigen specific antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components.
2. Peroxidase Conjugated goat anti-human IgG is added to the wells and the plate is incubated. The Conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unreacted Conjugate.

3. The microwells containing immobilized peroxidase Conjugate are incubated with peroxidase Substrate Solution. Hydrolysis of the Substrate by peroxidase produces a color change. After a period of time the reaction is stopped and the color intensity of the solution is measured photometrically. The color intensity of the solution depends upon the antibody concentration in the original test sample.

TEST SYSTEM COMPONENTS

Materials Provided:

Each Test System contains the following components in sufficient quantities to perform the number of tests indicated on the packaging label. **NOTE:** The following components contain Sodium Azide as a preservative at a concentration of <0.1% (w/v): Controls, Calibrator, and SAVe Diluent®.

Kit Component	Quantity Σ96	Quantity Σ480	Description
PLATE	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated VZV antigen (strain VZ-10). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ	1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgG (Fc chain specific). 15mL, white-capped bottle. Ready to use.
CTRL +	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial. 21X concentrate.
CAL	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial. 21X concentrate.
CTRL -	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial. 21X concentrate.
DIL SPE	1	4	SAVe Diluent®: 30mL, green-capped, bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Ready to use**. NOTE: The SAVe Diluent® will change color when combined with serum.**
SOLN TMB	1	5	TMB: 15mL, amber-capped, amber bottle containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN STOP	1	3	Stop Solution: 15mL, red-capped bottle containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASH 10X	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle containing a 10X concentrated phosphate-buffered-saline and Tween-20 solution (blue solution). **NOTE: 1X solution will have a pH of 7.2 ± 0.2.**

NOTE: The following components are not kit lot number dependent and may be used interchangeably between ELISA kits so long as the component product number is the same: TMB, Stop Solution, and Wash Buffer. SAVe Diluent® may be used interchangeably with any ELISA kit utilizing product number 005CC.

MATERIALS REQUIRED BUT NOT PROVIDED

1. ELISA microwell reader capable of reading at a wavelength of 450nm. **NOTE: Use of a single (450nm), or dual (450/620 - 650nm), wavelength reader is acceptable. Dual wavelength is preferred, as the additional reference filter has been determined to reduce potential interference from anomalies that may absorb light.**
2. Pipettes capable of accurately delivering 10 - 200µL.
3. Multichannel pipette capable of accurately delivering 50 - 200µL.
4. Reagent reservoirs for multichannel pipettes.
5. Wash bottle or microwell washing system.
6. Distilled or deionized water.
7. One-liter graduated cylinder.
8. Serological pipettes.
9. Disposable pipette tips.

10. Paper towels.
11. Laboratory timer to monitor incubation steps.
12. Disposal basin and disinfectant (i.e., 10% household bleach - 0.5% sodium hypochlorite).

STORAGE CONDITIONS

2°C – 8°C	Coated Microwell Strips: Immediately reseal extra strips with desiccant and return to proper storage. After opening, strips are stable for 60 days, as long as the indicator strips on the desiccant pouch remain blue.
	Conjugate – DO NOT FREEZE.
	Unopened Kit, Calibrator, Positive Control, Negative Control, TMB, Sample Diluent.
2°C – 25°C	Stop Solution: 2 – 25 °C Wash Buffer (1X): 20 - 25°C for up to 7 days, 2 - 8°C for 30 days Wash Buffer (10X): 2 - 25°C

PRECAUTIONS

1. For *In Vitro* diagnostic use.
2. Follow normal precautions exercised in handling laboratory reagents. In case of contact with eyes, rinse immediately with plenty of water and seek medical advice. Wear suitable protective clothing, gloves, and eye/face protection. Do not breathe vapor. Dispose of waste observing all local, state, and federal laws.
3. The wells of the ELISA plate do not contain viable organisms. However, consider the strips **potentially biohazardous materials** and handle accordingly.
4. The Controls are **potentially biohazardous materials**. Source materials from which these products were derived were found negative for HIV-1 antigen, HBsAg and for antibodies against HCV and HIV by approved test methods. However, since no test method can offer complete assurance that infectious agents are absent, handle these products at the Biosafety Level 2 as recommended for any potentially infectious human serum or blood specimen in the Centers for Disease Control/National Institutes of Health manual "Biosafety in Microbiological and Biomedical Laboratories": Current Edition; and OSHA's Standard for Bloodborne Pathogens (18).
5. Adherence to the specified time and temperature of incubations is essential for accurate results. **All reagents must be allowed to reach room temperature (20 - 25°C) before starting the assay**. Return unused reagents to refrigerated temperature immediately after use.
6. Improper washing could cause false positive or false negative results. Be sure to minimize the amount of any residual wash solution; (e.g., by blotting or aspiration) before adding Conjugate or Substrate. Do not allow the wells to dry out between incubations.
7. The SAVe Diluent®, Controls, and Calibrator contain Sodium Azide at a concentration of <0.1% (w/v). Sodium Azide has been reported to form lead or copper azides in laboratory plumbing which may cause explosions on hammering. To prevent, rinse sink thoroughly with water after disposing of solution containing Sodium Azide.
8. The Stop Solution is TOXIC if inhaled, has contact with skin or if swallowed. It can cause burns. In case of accident or ill feelings, seek medical advice immediately.
9. The TMB Solution is HARMFUL. It is irritating to eyes, respiratory system and skin.
10. The Wash Buffer concentrate is an IRRITANT. It is irritating to eyes, respiratory system and skin.
11. Wipe the bottom of the plate free of residual liquid and/or fingerprints that can alter optical density (OD) readings.
12. Dilution or adulteration of these reagents may generate erroneous results.
13. Do not use reagents from other sources or manufacturers.
14. TMB Solution should be colorless, very pale yellow, very pale green, or very pale blue when used. Contamination of the TMB with Conjugate or other oxidants will cause the solution to change color prematurely. Do not use the TMB if it is noticeably blue in color.
15. Never pipette by mouth. Avoid contact of reagents and patient specimens with skin and mucous membranes.
16. Avoid microbial contamination of reagents. Incorrect results may occur.
17. Cross contamination of reagents and/or samples could cause erroneous results.
18. Reusable glassware must be washed and thoroughly rinsed free of all detergents.
19. Avoid splashing or generation of aerosols.
20. Do not expose reagents to strong light during storage or incubation.
21. Allowing the microwell strips and holder to equilibrate to room temperature prior to opening the protective envelope will protect the wells from condensation.

22. Collect the wash solution in a disposal basin. Treat the waste solution with disinfectant (i.e.: 10% household bleach - 0.5% Sodium Hypochlorite). Avoid exposure of reagents to bleach fumes.
23. Caution: Neutralize any liquid waste at an acidic pH before adding to a bleach solution.
24. Do not use ELISA plate if the indicator strip on the desiccant pouch has turned from blue to pink.
25. Do not allow the Conjugate to come in contact with containers or instruments that may have previously contained a solution utilizing Sodium Azide as a preservative. Residual amounts of Sodium Azide may destroy the Conjugate's enzymatic activity.
26. Do not expose any of the reactive reagents to bleach-containing solutions or to any strong odors from bleach-containing solutions. Trace amounts of bleach (sodium hypochlorite) may destroy the biological activity of many of the reactive reagents within this Test System.

SPECIMEN COLLECTION

1. ZEUS Scientific recommends that the user carry out specimen collection in accordance with CLSI document M29: Protection of Laboratory Workers from Infectious Disease (Current Edition).
2. No known test method can offer complete assurance that human blood samples will not transmit infection. Therefore, consider all blood derivatives potentially infectious.
3. Use only freshly drawn and properly refrigerated sera obtained by approved aseptic venipuncture procedures in this assay (15, 16). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera.
4. Store sample at room temperature for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2 - 8°C, for no longer than 48 hours. If a delay in testing is anticipated, store test sera at –20°C or lower. Avoid multiple freeze/thaw cycles which may cause loss of antibody activity and give erroneous results. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine stability criteria for its laboratory (19).

ASSAY PROCEDURE

1. Remove the individual components from storage and allow them to warm to room temperature (20 - 25°C).
2. Determine the number of microwells needed. Allow for six Control/Calibrator determinations (one Reagent Blank, one Negative Control, three Calibrators and one Positive Control) per run. Run a Reagent Blank on each assay. Check software and reader requirements for the correct Controls/Calibrator configurations. Return unused strips to the resealable pouch with desiccant, seal, and return to storage between 2 - 8°C.

EXAMPLE PLATE SET-UP		
	1	2
A	Blank	Patient 3
B	Negative Control	Patient 4
C	Calibrator	Etc.
D	Calibrator	
E	Calibrator	
F	Positive Control	
G	Patient 1	
H	Patient 2	

3. Prepare a 1:21 dilution (e.g.: 10µL of serum + 200µL of SAVe Diluent®) of the Negative Control, Calibrator, Positive Control, and each patient serum. **NOTE: The SAVe Diluent® will undergo a color change confirming that the specimen has been combined with the diluent.**
4. To individual wells, add 100µL of each diluted Control, Calibrator and patient specimen. Ensure that the samples are properly mixed. Use a different pipette tip for each sample.
5. Add 100µL of SAVe Diluent® to well A1 as a Reagent Blank. Check software and reader requirements for the correct Reagent Blank well configuration.
6. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
7. Wash the microwell strips 5 times.
   a. **Manual Wash Procedure**:
      1. Vigorously shake out the liquid from the wells.
      2. Fill each microwell with Wash Buffer. Make sure no air bubbles are trapped in the wells.
      3. Repeat steps 1. and 2. for a total of 5 washes.

4. Shake out the wash solution from all the wells. Invert the plate over a paper towel and tap firmly to remove any residual wash solution from the wells. Visually inspect the plate to ensure that no residual wash solution remains. Collect wash solution in a disposable basin and treat with disinfectant at the end of the day's run.

b. **Automated Wash Procedure**:
If using an automated microwell wash system, set the dispensing volume to 300 - 350µL/well. Set the wash cycle for 5 washes with no delay between washes. If necessary, the microwell plate may be removed from the washer, inverted over a paper towel and tapped firmly to remove any residual wash solution from the microwells.

8. Add 100µL of the Conjugate to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
9. Incubate the plate at room temperature (20 - 25°C) for 25 ± 5 minutes.
10. Wash the microwells by following the procedure as described in step 7.
11. Add 100µL of TMB to each well, including the Reagent Blank well, at the same rate and in the same order as the specimens.
12. Incubate the plate at room temperature (20 - 25°C) for 10 - 15 minutes.
13. Stop the reaction by adding 50µL of Stop Solution to each well, including the Reagent Blank well, at the same rate and in the same order as the TMB. Positive samples will turn from blue to yellow. After adding the Stop Solution, tap the plate several times to ensure that the samples are thoroughly mixed.
14. Set the microwell reader to read at a wavelength of 450nm and measure the optical density (OD) of each well against the Reagent Blank. Read the plate within 30 minutes of the addition of the Stop Solution.

ABBREVIATED TEST PROCEDURE

1. Dilute Serum 1:21.
2. Add diluted sample to microwell - 100µL/well.
3. ⟶ *Incubate 25 ± 5 minutes.*
4. Wash.
5. Add Conjugate - 100µL/well.
6. ⟶ *Incubate 25 ± 5 minutes.*
7. Wash.
8. Add TMB - 100µL/well.
9. ⟶ *Incubate 10 - 15 minutes.*
10. Add Stop Solution - 50µL/well - Mix.
11. READ within 30 minutes.

QUALITY CONTROL

1. Each time the assay is performed, the Calibrator must be run in triplicate. A Reagent Blank, Negative Control, and Positive Control must also be included.
2. Calculate the mean of the three Calibrator wells. If any of the three values differ by more than 15% from the mean, discard that value and calculate the mean using the remaining two wells.
3. The mean OD value for the Calibrator, Positive Control, and Negative Control should fall within the following ranges:

	OD Range
Negative Control	≤0.250
Calibrator	≥0.300
Positive Control	≥0.500

   a. The OD of the Negative Control divided by the mean OD of the Calibrator should be ≤0.9.
   b. The OD of the Positive Control divided by the mean OD of the Calibrator should be ≥1.25.
   c. If the above conditions are not met the test should be considered invalid and should be repeated.
4. The Positive Control and Negative Control are intended to monitor for substantial reagent failure, but will not ensure precision at the assay Cutoff.
5. Additional Controls may be tested according to guidelines or requirements of local, state, and/or federal regulations or accrediting organizations.
6. Refer to CLSI document C24: Statistical Quality Control for Quantitative Measurement Procedures for guidance on appropriate QC practices.

INTERPRETATION OF RESULTS

1. **Calculations:**
   a. *Correction Factor*: The manufacturer determined a Cutoff OD Value for positive samples and correlated it to the Calibrator. The Correction Factor (CF) allows for the determination of the Cutoff Value for positive samples. It will also correct for slight day-to-day variations in test results. The Correction Factor is determined for each lot of components and is printed on the Component Label located in the Test System box.
   b. *Cutoff OD Value*: To obtain the Cutoff OD Value, multiply the CF by the mean OD of the Calibrator determined above. *(CF x Mean OD of Calibrator = Cutoff OD Value)*
   c. *Index Values/OD Ratios*: Calculate the Index Value/OD Ratio for each specimen by dividing its OD Value by the Cutoff OD from step b.

Example:	Mean OD of Calibrator	=	0.793
	Correction Factor (CF)	=	0.25
	Cutoff OD	=	0.793 x 0.25 = 0.198
	Unknown Specimen OD	=	0.432
	Specimen Index Value/OD Ratio	=	0.432/0.198 = 2.18

2. **Interpretations:** Index Values/OD Ratios are interpreted as follows.

	Index Value/OD Ratio
Negative Specimens	≤0.90
Equivocal Specimens	0.91 to 1.09
Positive Specimens	≥1.10

   a. An OD ratio ≤0.90 indicates no significant amount of IgG antibodies to VZV detected. A non-reactive result indicates no current or previous infection with VZV. Such patients are presumed to be non-immune and are therefore susceptible to a primary infection.
   b. An OD ratio ≥1.10 indicates that IgG antibodies specific to VZV were detected. A reactive test result indicates a past or current infection with VZV. A reactive result indicates that the patient in question should be considered immune to VZV primary infection. The results of this test system are qualitative. The magnitude of the ratio for positive specimens may not correlate with antibody titer. If testing a particular sample occurs early during primary infection, no detectable IgG may be evident. If VZV infection is suspected, a second sample should be taken at least fourteen days later.
   c. Specimens with OD ratio values in the equivocal range (0.91 - 1.09) should be retested in duplicate. Report any two of the three results which agree. Evaluate repeatedly equivocal specimen by an alternate serologic procedure and/or re-evaluate by drawing another sample one to three weeks later.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis on the basis of Anti-VZV IgG results alone. Interpret test results for anti-VZV in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. Interpret positive test results from cord blood or neonates with caution.
3. A reactive test result in immunocompromised patients may not be indicative of prior infection with Varicella virus. Interpret assay results from recent blood products with caution.
4. ZEUS Scientific did not establish performance characteristics with individuals vaccinated with VZV (OKA Strain).

EXPECTED RESULTS

1. Population studies using diagnostic tests for antibody analysis indicate that most individuals have had previous infections with VZV by the time they are 20 years old (17).
2. The clinical study for this product included 200 random, routine specimens which were sent to a reference laboratory for VZV serology. With respect to this population, 35 of the 200 specimens (17.5%) were negative, 161 of the 200 (80.5%) were positive, and four of the 200 (2%) were equivocal. Below appears a frequency distribution from the study described above.

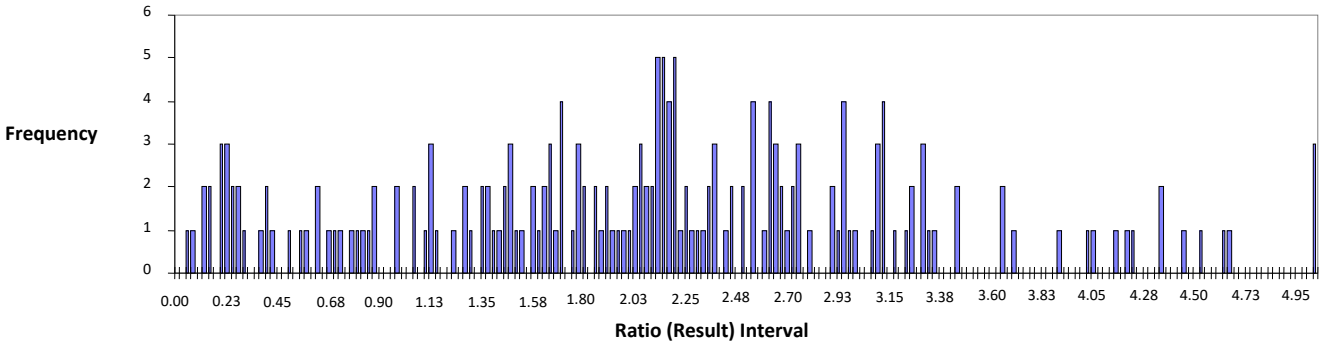

Figure 1: Frequency Distribution of 200 Routine VZV Serology Specimens.

PERFORMANCE CHARACTERISTICS

1. **Comparative Study:**

A comparative study was conducted to demonstrate the equivalence of the Anti-VZV IgG to another ELISA test system currently in commercial distribution in a two-site clinical investigation. Briefly, there were a total of 241 specimens tested: 121 at Site One, and 120 at Site Two. Specimens tested at Site One included 100 samples sent to a reference lab for routine VZV serology, and 21 specimens previously characterized as VZV IgG negative. Specimens tested at Site Two included 100 specimens sent to a reference lab for routine VZV serology, and 20 specimens previously characterized as VZV IgG negative. Tables 1 and 2 summarize the results of this comparative study.

Table 1: Calculation of Relative Sensitivity, Specificity, and Agreement; Clinical Site One

		Anti-VZV IgG Results*			
		Positive	Negative	Equivocal	Total
Commercial ELISA Results	Positive	78	3	3*	84
	Negative	2	34	0	36
	Equivocal	1*	0	0	1
	Total	81	37	3	121

Relative Sensitivity = 78/81 = 96.3% 95% Confidence Interval** = 92.2 to 100 %

Relative Agreement = 112/117 = 95.7% 95% Confidence Interval** = 92.1 to 99.4 %

Relative Specificity = 34/36 = 94.4% 95% Confidence Interval** = 90.0 to 100 %

*Samples were equivocal on the Anti-VZV IgG. Repeat testing resulted in repeatedly equivocal results. One specimen was found to be repeatedly equivocal on the commercial assay. These four specimens were excluded from results. ** 95% confidence intervals calculated using the exact method.

Table 2: Calculation of Relative Sensitivity, Specificity, and Agreement; Clinical Site Two

		Anti-VZV IgG Results			
		Positive	Negative	Equivocal	Total
Commercial ELISA Results	Positive	77	3	2	82
	Negative	3	34	0	37
	Equivocal	0	1***	0	1
	Total	80	38	2	120

Relative Sensitivity = 77/80 = 96.3% 95% Confidence Interval** = 92.1 to 100 %

Relative Agreement = 111/117 = 94.9% 95% Confidence Interval** = 90.9 to 98.9 %

Relative Specificity = 34/37 = 91.9% 95% Confidence Interval** = 83.1 to 100 %

**95% confidence intervals calculated using the exact method.

***Two specimens were equivocal on the Anti-VZV IgG Results, and one specimen was equivocal on the commercial system. The specimens were not repeat tested due to insufficient volume. These three specimens were not included in any calculations.

2. **Reproducibility**

Reproducibility was evaluated as outlined in document number EP5: Evaluation of Precision Performance of Clinical Chemistry Devices - Second Edition, as published by the Clinical Laboratory Standards Institute (CLSI), Villanova, PA. Reproducibility studies were conducted at both of the clinical sites using the same specimens. Briefly, eight specimens were tested, two relatively strong positive specimens, two specimens closer to the cutoff optical density (one weakly positive, and one a high negative), two negative specimens and the Test System's Negative and Positive Controls. On each day of testing, each of the eight specimens were assayed

in duplicate. Also, on each day of testing, the assay was performed twice, once in the morning and once in the afternoon for a total of four replicates for each specimen daily. Site One conducted this reproducibility study for a 20 day period for a total of 80 observations for each of the eight panel members. Site Two conducted the investigation for a total of ten days, resulting in 40 observations for each sample. Table 3 shows a summary of the study.

Table 3. Summary of Precision Testing Conducted at Clinical Sites One and Two

Specimen	Site	Mean Ratio	Result	SWR*	ST**	Days	Total Observations	Overall % CV
VG-1	1	3.124	Positive	0.132	0.167	20	80	5.35
	2	4.537		0.242	0.518	10	40	11.43
VG-5	1	3.144	Positive	0.059	0.141	20	80	4.47
	2	4.370		0.176	0.457	10	40	10.46
VG-3	1	1.531	Moderate	0.057	0.087	20	80	5.69
	2	2.148	Positive	0.164	0.324	10	40	15.06
VG-4	1	0.731	High	0.023	0.040	20	80	5.48
	2	0.606	Negative	0.057	0.085	10	40	14.09
VG-9	1	0.152	Negative	0.012	0.022	20	80	N/A
	2	0.010		0.022	0.026	10	40	
VG-10	1	0.198	Negative	0.014	0.022	20	80	N/A
	2	0.067		0.030	0.061	10	40	
Positive Control	1	3.851	Positive	0.188	0.218	20	80	5.65
	2	2.114		0.226	0.312	10	20	14.77
Negative Control	1	0.197	Negative	0.009	0.025	20	80	N/A
	2	0.022		0.025	0.024	10	40	
*Point estimate of within run precision standard deviation								
** Point estimate of total precision standard deviation.								

3. **Cross Reactivity**

Cross reactivity studies were performed to assess interference in the Anti-VZV IgG using sera which were negative for antibodies to VZV, and which demonstrated antibodies to Rubella, CMV, HSV-1, HSV-2, EBV-VCA, EBNA-1, Measles, and Mumps. The results of this study are summarized in Table 4 below. All Test Systems utilized were manufactured by ZEUS Scientific, Inc. for commercial distribution. This study resulted in no detectable cross reactivity with these various IgG antibodies, and the Anti-VZV IgG.

Table 4: Cross Reactivity Analysis

Specimen	B12	C10	E9	E8	D5	E4	F3	F2
VZV IgG Result	0.14	0.16	0.58	0.74	0.71	0.52	0.63	0.45
Interpretation	(-)	(-)	(-)	(-)	(-)	(-)	(-)	(-)
Rubella Result	0.81	0.89	1.98	1.30	3.80	3.25	2.21	1.31
Interpretation	(-)	(-)	(+)	(+)	(+)	(+)	(+)	(+)
CMV Result	2.40	3.55	0.22	0.24	0.61	1.98	2.73	0.15
Interpretation	(+)	(+)	(-)	(-)	(-)	(+)	(+)	(-)
HSV-1 Result	1.42	1.67	2.07	1.02	0.84	1.77	1.32	2.32
Interpretation	(+)	(+)	(+)	(±)	(-)	(+)	(+)	(+)
HSV-2 Result	1.45	0.63	1.02	3.10	0.71	1.04	0.50	1.07
Interpretation	(+)	(-)	(±)	(+)	(-)	(+)	(-)	(±)
VCA Result	2.49	3.84	2.80	4.00	4.27	3.15	1.69	1.22
Interpretation	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
EBNA Result	4.51	5.66	2.46	6.52	1.94	3.28	5.29	4.43
Interpretation	(+)	(+)	(+)	(+)	(+)	(+)	(+)	(+)
Measles Result	1.55	1.46	0.94	0.88	1.64	1.26	1.42	1.94
Interpretation	(+)	(+)	(±)	(-)	(+)	(+)	(+)	(+)
Mumps Result	2.65	3.65	2.78	0.90	2.34	5.02	2.77	4.12
Interpretation	(+)	(+)	(+)	(-)	(+)	(+)	(+)	(+)
Note: For all Test Systems indicated above: ≥1.10 is reactive (+) ≤0.90 is negative (-) 0.91-1.09 is equivocal (±)								

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GLOSSARY OF SYMBOLS

The following symbols **may** have been used in the labelling of this product or products associated with this product.

Symbol	Description	Symbol	Description
	Manufacturer		Keep away from sunlight
IVD	*In vitro* diagnostic medical device	PLATE	Plate
REF	Catalogue number	CONJ	Conjugate
Σ n	Sufficient for *n* tests	CTRL +	Positive Control
LOT	Batch code	CTRL -	Negative Control
	Use by	CAL	Calibrator
	Temperature limitation	DIL SPE	Sample Diluent
CONT	Contents	SOLN TMB	TMB
UDI	Unique Device Identifier	SOLN STOP	Stop Solution
	Consult the warnings and precautions	WASH 10X	Wash Buffer Concentrate (10X)
	Consult electronic instructions for use	EN	English
	Store in the upright position	Made in the USA	Made in the USA
RX Only	Applicable for U.S.A: Prescription *in vitro* diagnostic product		Corrosive
	Hazardous Communication	EC REP	European Commission Authorized Representative
CE	Conformity with Directive 98/79		

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