VZV IgM Test System

REF 9Z9331M/SM9Z9331M/9Z9331MB

IVD

Rx Only

INTENDED USE

The ZEUS ELISA Varicella-Zoster Virus (VZV) IgM Test System is intended for the qualitative detection of VZV IgM antibody in human sera as an aid in the diagnosis of primary infection or reactivation. The assay performance in detecting antibodies to VZV in individuals vaccinated with the FDA licensed VZV vaccine is unknown. The user of this assay is responsible for establishing the performance characteristics with VZV vaccinated individuals. The assay performance in detecting antibodies to VZV in cord blood and neonates has not been established.

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. Often, 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 that 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. It 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 ZEUS ELISA VZV IgM Test System is designed to detect IgM class antibodies to VZV in human sera. Creation of sensitized wells of plastic microwell strips occurred by passive adsorption with VZV antigen. The test procedure involves three incubation steps:

1. Test sera are diluted with the Sample Diluent provided. The Sample Diluent contains anti-human IgG that is intended to bind IgG and Rheumatoid Factor to prevent non-specific binding to the immobilized antigen. During sample incubation any antigen specific IgM 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 IgM is added to the wells and the plate is incubated. The Conjugate will react with IgM antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound 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.

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 Sample Diluent.**

Component			Σ 96	Σ 480	
PLATE		1.	1	5	Plate: 96 wells configured in twelve, 1x8-well, strips coated with inactivated VZV antigen (strain-Ellen). The strips are packaged in a strip holder and sealed in an envelope with desiccant.
CONJ		2.	1	5	Conjugate: Conjugated (horseradish peroxidase) goat anti-human IgM (μ chain specific) in 15mL, white-capped bottle(s). Ready to use.
CONTROL	+	3.	1	2	Positive Control (Human Serum): 0.35mL, red-capped vial(s).
CAL		4.	1	4	Calibrator (Human Serum): 0.5mL, blue-capped vial(s).
CONTROL	-	5.	1	2	Negative Control (Human Serum): 0.35mL, green-capped vial(s).
DIL	SPE	6.	1	4	Sample Diluent: 30mL, green-capped bottle(s) containing Tween-20, bovine serum albumin and phosphate-buffered-saline. Purple Solution. Ready to use.
SOLN	TMB	7.	1	5	TMB: 15mL, amber-capped, amber bottle(s) containing 3, 3', 5, 5' - tetramethylbenzidine (TMB). Ready to use.
SOLN	STOP	8.	1	3	Stop Solution: 15mL, red-capped bottle(s) containing 1M H_2SO_4, 0.7M HCl. Ready to use.
WASHBUF	10X	9.	1	5	Wash Buffer Concentrate (10X): Dilute 1 part concentrate + 9 parts deionized or distilled water. 100mL, clear-capped bottle(s) 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.**

NOTES:

1. **The following components are not Test System Lot Number dependent and may be used interchangeably with the ZEUS ELISA Test Systems: TMB, Stop Solution, and Wash Buffer.**
2. **Test System also contains a Component Label containing lot specific information inside the Test System box.**

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 (15).
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 Sample 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 upon 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.

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 remains blue.
	Conjugate – DO NOT FREEZE.
	Unopened Test System, 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

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 (16, 17). Do not use if there are any added anticoagulants or preservatives. Avoid using hemolyzed, lipemic, or bacterially contaminated sera and samples that contain high levels of IgG. High levels of IgG have been shown to reduce reactivity to VZV IgM antibody.
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 (18).

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 Sample Diluent) of the Negative Control, Calibrator, Positive Control, and each patient serum.
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 Sample 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 in each assay.
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 IgM antibodies to VZV detected. A negative result indicates no active infection with VZV. However, specimens taken too early during a primary infection may not have detectable levels of IgM antibody. If a primary infection is suspected, another specimen should be taken within seven days and tested concurrently in the same assay with the original specimen to look for seroconversion.
   b. An OD ratio ≥1.10 indicates that IgM antibodies specific to VZV were detected. A positive value indicates a primary or reactivated infection with VZV. Such individuals are presumed to be at risk of transmitting VZV infection.
   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. If the second specimen is positive, consider the patient to have an active VZV infection.

LIMITATIONS OF THE ASSAY

1. Do not make a diagnosis based on ZEUS ELISA VZV IgM Test System results alone. Interpret test results for anti-VZV in conjunction with the clinical evaluation and the results of other diagnostic procedures.
2. This assay is not intended for use in blood donor populations.
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. The performance characteristics with individuals vaccinated with VZV (OKA Strain) have not been established.
5. The anti-IgG absorbent in the Sample Diluent has been found to functionally remove ≥ 13.9 mg/mL IgG from human serum. Patients with an IgG level exceeding 14 mg/mL may require additional treatment to neutralize all IgG. Excessively high levels of IgG have been shown to reduce reactivity to VZV IgM antibody.
6. Potential cross-reactivity of this assay with specimens from individuals infected with HSV-1 or HSV-2 has not been evaluated.

EXPECTED RESULTS

Population studies using diagnostic tests for antibody analysis indicate that most individuals have had previous infections with VZV by the time that they are 20 years old (18). The clinical study for this product included 302 random, routine specimens that were submitted for VZV antibody testing. Two samples were submitted with sex unknown. Table 1 summarizes the demographics. The data obtained from this clinical study is provided in Table 2.

Table 1: Demographics

	Sample (N)	Submitted for	From Region	Sex (Male: Female)	Median Age	Pediatric Samples	Women of Childbearing Age
Site One	131	VZV Antibody	NE	17 : 114*	32.1	2	103
Site Two	47	VZV Antibody	NE	8 : 38*	36.9	2	20
Site Three	124	VZV Antibody	West	39 : 84	34.6	13	62
Total	**302**			**64 : 236**	**34.5**	**17**	**185**

* Patient submitted with sex unknown.

Table 2: Expected Values/ Reference Ranges

Age	Specimen Group	VZV IgM Negative	VZV IgM Positive	VZV IgM Equivocal	Invalid
1 - 9	Prospective Males	1	4		
	Prospective Females		3		
10 - 19	Prospective Males	1	10		
	Prospective Females		19	1	
20 - 29	Prospective Males		13		
	Prospective Females		85	2	
	Sex Unknown		1		
30 - 39	Prospective Males		14		
	Prospective Females		64		
	Sex Unknown		1		
40 - 49	Prospective Males		7		
	Prospective Females		26	1	
50 - 59	Prospective Males		7		
	Prospective Females	1	16		
60 - 69	Prospective Males		4		
	Prospective Females	2	10		
70+	Prospective Males		3		
	Prospective Females	1	4	1	
Totals	Prospective Males	2	62		

	Prospective Females	4	227	5	
	Sex Unknown		2		
	Sub-Total	6	291	5	0
	Total	302			

PERFORMANCE CHARACTERISTICS

1. **Comparative Study**

A comparative study was conducted to demonstrate the equivalence of the ZEUS ELISA VZV IgM Test System to another VZV IgM ELISA test system currently in commercial distribution. Performance evaluation occurred using a three-site clinical investigation. Briefly, there were 338 samples tested: 131 at Site One, 53 at Site Two and 154 at Site Three. Samples at Site One were submitted for VZV antibody testing. Samples at Site Two included 47 specimens submitted for routine VZV antibody testing and six specimens which were previously characterized as positive for VZV IgM antibody. Samples at Site Three included 124 routine specimens submitted for VZV antibody testing and 30 previously characterized positive specimens. The results of this comparative study have been summarized in the following tables, one depicting prospective specimens and one with both prospective and retrospective samples:

Table 3: Prospective Samples Combined Sites

		Commercial ELISA Results			
		Positive	Negative	Equivocal	Total
ZEUS ELISA VZV IgM Test System Results	Positive	6	4	2	12
	Negative		281		281
	Equivocal		7	2	9
	Total	6	292	4	302

Positive % Agreement = 6/6 = 100% 95% Confidence Interval** =54.1% to 100%
Negative % Agreement = 281/294 = 95.6% 95% Confidence Interval** =96.2% to 97.6%

Table 4: Prospective and Retrospective Samples Combined Sites

		Commercial ELISA Results			
		Positive	Negative	Equivocal	Total
ZEUS ELISA VZV IgM Test System Results	Positive	38	4	4	46
	Negative		282		282
	Equivocal	1	7	2	10
	Total	39	293	6	338

Positive % Agreement = 38/39 = 97.4% 95% Confidence Interval** =86.5% to 99.9%
Negative % Agreement = 282/297 = 94.9% 95% Confidence Interval** = 91.8% to 97.1%
**95% Confidence Intervals calculated using the exact method.

2. **Reproducibilty**

Reproducibility was evaluated as outlined in document number EP5: Evaluation of Precision Performance of Clinical Chemistry Devices – Second Edition, as published by CLSI. Reproducibility studies were conducted at all three sites using the same specimens. Six specimens were tested; two strong positive specimens, two specimens close to the cutoff optical density and two negative specimens. On each day of testing, each specimen was assayed in eight replicate wells. This was done for a total of three days. Table 5 shows a summary of the precision testing conducted at the three sites:

Table 5: Summary of Reproducibility Testing

		Site 1			Site 2			Site 3			Inter-Assay Precision			Between Site
Sample		Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Site 1	Site 2	Site 3	
1	Mean	3.38	3.52	3.50	3.60	4.21	3.69	3.43	3.54	3.38	3.47	3.84	3.45	3.6
	StD	0.10	0.06	0.08	0.03	0.12	0.06	0.08	0.10	0.07	0.10	0.29	0.10	0.3
	% CV	2.8	1.8	2.2	1.0	2.9	1.5	2.3	2.7	2.1	0.03	0.07	0.03	7.1
2	Mean	2.89	2.90	2.86	2.96	3.18	2.92	2.99	2.97	2.95	2.90	3.02	2.97	3.0
	StD	0.03	0.02	0.09	0.05	0.04	0.06	0.03	0.08	0.07	0.10	0.13	0.06	0.1
	% CV	1.0	0.8	3.0	1.6	1.4	2.1	1.1	2.7	2.3	0.02	0.04	0.02	3.4
3	Mean	0.26	0.23	0.24	0.34	0.40	0.33	0.19	0.20	0.18	0.20	0.36	0.19	0.3
	StD	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.03	0.001	0.1
	% CV	5.0	2.3	3.4	2.1	2.9	3.8	3.8	7.5	8.0	0.07	0.10	0.07	28.1
4	Mean	0.13	0.11	0.10	0.15	0.14	0.13	0.12	0.13	0.10	0.10	0.14	0.11	0.1
	SD	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.00	0.05	0.03	0.0
	% CV	3.6	3.6	2.6	5.0	4.0	7.1	7.5	7.3	8.5	0.11	0.07	0.13	14.0
5	Mean	0.91	0.89	0.94	0.82	0.89	0.91	0.95	0.91	0.93	0.90	0.87	0.93	0.9
	StD	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.00	0.05	0.03	0.0
	% CV	1.5	2.5	2.4	2.8	1.5	1.8	2.6	2.8	1.7	0.03	0.05	0.03	4.6
6	Mean	0.90	0.89	0.88	0.73	0.87	0.91	1.00	0.93	0.91	0.90	0.84	0.95	0.9
	StD	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.02	0.03	0.00	0.08	0.05	0.1
	% CV	3.0	2.	0.9	3.3	2.4	1.4	3.3	2.5	3.4	0.02	0.10	0.05	8.0

3. **Cross Reactivity**

Studies were performed to assess cross reactivity in the ZEUS ELISA VZV IgM Test System using sera that contained IgM antibodies to EBV, CMV, Mumps, Rheumatoid factor, Lyme, Toxoplasma, Rubella and Measles. All Test Systems utilized in this study were manufactured by ZEUS Scientific, Inc. for commercial distribution. This study produced no detectable cross reactivity with these various antibodies.

Table 6: Cross Reactivity

Disease Condition	Number Tested	Number Positive or Equivocal
EBV IgM	10	0/10
CMV IgM	10	0/10
Mumps	10	0/10
RF IgM	10	0/10
Lyme IgM	10	0/10
Toxoplasma IgM	10	0/10
Rubella IgM	10	0/10
Measles IgM	10	0/10

Caution: Cross reactivity of this assay with specimens containing antibody to HSV-1 and HSV-2 virus has not been determined.

4. **Interfering Substances**

The effect of potential interfering substances on positive sample results generated using the ZEUS ELISA VZV IgM Test System was evaluated with the following possible interferants: bilirubin, albumin, IgG, cholesterol, triglyceride, hemoglobin and intralipids. Three specimens, tested for VZV IgM antibody, were spiked with twice the normal level of the possible interferant and retested using the ZEUS ELISA VZV IgM Test System. Some elevation of signal in the presence of excess hemoglobin was noted. Reduction of signal in the presence of excessively high IgG was noted. In all cases, the qualitative outcome of the three samples remained unchanged.

Table 7: Interfering Substances (Note: all results are reported as Index Values)

	Spiked Level	Sample 1		Sample 2		Sample 3	
		Positive	% Positive Signal	Equivocal	% Positive Signal	Negative	% Control Signal
Control – PBS	N/A	3.67	N/A	0.88	N/A	0.07	N/A
Control – Ethanol	N/A	3.59	N/A	0.82	N/A	0.07	N/A
Bilirubin	Low	3.78	103.16%	0.93	105.33%	0.08	123.53%
Bilirubin	High	3.59	97.93%	0.90	101.7%	0.06	92.65%
Albumin	Low	3.63	99.05%	0.91	103.63%	0.06	88.24%
Albumin	High	3.82	104.01%	0.89	100.45%	0.07	108.82%
IgG	Low	2.71	69.6%	0.79	83.0%	0.10	245.0%
IgG	High	1.98	48.6%	0.51	56.70%	0.16	400.0%
Cholesterol	Low	3.50	97.63%	0.88	107.6%	0.07	100.0%
Cholesterol	High	3.60	100.33%	0.88	107.6%	0.07	102.82%
Triglycerides	Low	3.80	105.94%	0.87	106.99%	0.07	100.0%
Triglycerides	High	3.79	105.61%	0.88	107.6%	0.07	92.96%
Hemoglobin	Low	3.77	102.81%	0.94	106.58%	0.14	201.47%
Hemoglobin	High	4.06	110.66%	0.97	109.64%	0.11	167.65%
Intralipid	Low	3.77	102.73%	0.87	98.53%	0.08	120.59%
Intralipid	High	3.62	98.66%	0.87	98.75%	0.07	98.53%
Control	N/A	3.66	N/A	0.89	N/A	0.06	N/A

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