510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE

A. 510(k) Number:

K070317

B. Purpose for Submission:

New device clearance

C. Measurand:

IgM antibodies to human Varicella-Zoster virus (VZV)

D. Type of Test:

Qualitative Elisa

E. Applicant:

Zeus Scientific, Inc

F. Proprietary and Established Names:

Zeus Scientific Varicella-Zoster IgM Test System

G. Regulatory Information:

Product Code	Classification	Regulation Section	Panel
LFY	Class II	Varicella-zoster virus serological reagents (21 CFR 866.3900)	83 Microbiology

H. Intended Use:

1. Intended use(s):

   The Zeus Scientific Varicella-Zoster (VZV) IgM ELISA test system is intended for the qualitative detection of IgM antibody to Varicella-Zoster virus in human serum as an aid in the diagnosis of primary infection or

reactivation.

The assaay 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.

2. Indication(s) for use:

   The Zeus Scientific Varicella-Zoster (VZV) IgM ELISA test system is intended for the qualitative detection of IgM antibody to Varicella-Zoster virus in human serum as an aid in the diagnosis of primary infection or reactivation.

3. Special conditions for use statement(s):

   Prescription use only

4. Special instrument requirements:

   Not applicable

I. Device Description:

Enzyme linked immunosorbent assay

J. Substantial Equivalence Information:

a) Predicate device name(s):
Trinity Biotech Captia™ VZV IgM ELISA

b) Predicate K number(s):

Comparison with predicate:

Characteristic	**Zeus Scientific VZV IgM ELISA**	**Predicate ELISA**
Use	For in vitro diagnostic use only	For in vitro diagnostic use only
Use	intended for the detection of IgM antibody to Varicella-zoster virus in human serum as an aid in the diagnosis of primary infection or reactivation	intended for the detection of IgM antibody to Varicella-zoster virus in human serum as an aid in the diagnosis of primary infection or reactivation

Assay	Immunoassay	Immunoassay
Detection Method	Colormetric	Colormetric
Solid Phase	Polystyrene 96 well plate	Polystyrene 96 well plate
Antigen Used	Varicella-zoster virus, Ellen strain from Ross Southern Diagnostics	Varicella-zoster virus, Ellen strain
Specimen Tested	Human Serum	Human Serum
Controls	One PC and one NC	One PC and one NC
Calibration	Includes a calibrator (serum sample)	Includes a calibrator (serum sample)
Analyte Measured	Human IgM	Human IgM
Sample Dilution	1:21 in SAVe Diluent	1:41 in Diluent
Sample Incubation Period	25 +/- 5 Minutes at room temperature	20 +/- 2 minutes at room temperature
Post Sample Wash	5x wash (dispense / aspirate)	5x wash (dispense / aspirate)
Conjugate	Goat anti-human IgM; *u* chain specific	Goat anti-human IgM; *u* chain specific
Conjugate Label	Horse radish peroxidase	Horse radish peroxidase
Conjugate Incubation	25 +/- 5 Minutes at room temperature	20 +/- 2 minutes at room temperature
Post Conjugate Wash	5x wash (dispense / aspirate)	5x wash (dispense / aspirate)

Substrate	TMB	TMB
Reading	Read the color change (optical density) of the wells.	Read the color change (optical density) of the wells.
Data Points	Read one OD value for each control and sample	Read one OD value for each control and sample
Math	Single point curve	Single point curve
Scale	Calculate the index value of unknown samples by comparing their OD to the cut off OD	Calculate the index value of unknown samples by comparing their OD to the cut off OD
Interpretation Criteria	Negative is <=0.90, Positive is >= 1.10 and Equivocal is 0.91 to 1.09	Negative is <=0.90, Positive is >= 1.10 and Equivocal is 0.91 to 1.09

K. Standard/Guidance Document Referenced (if applicable):

Not applicable

L. Test Principle:

The Zeus VZV IgM ELISA test is designed to detect IgM class antibodies to VZV in human sera. Wells of plastic microwell strips are sensitized by passive absorption 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 the IgG and rheumatoid factor present in the patient specimen to prevent non-specific binding of the IgG and rheumatoid factor to the immobilized VZV 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 (μ chain specific) 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. The color intensity of the solution depends upon the antibody concentration in the original test sample.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/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 and Laboratory Standards Institute (CLSI), Villanova,PA. 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 cut off 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. The following tables summarize the precision testing conducted at the three sites:

Reproducibility Testing Summary

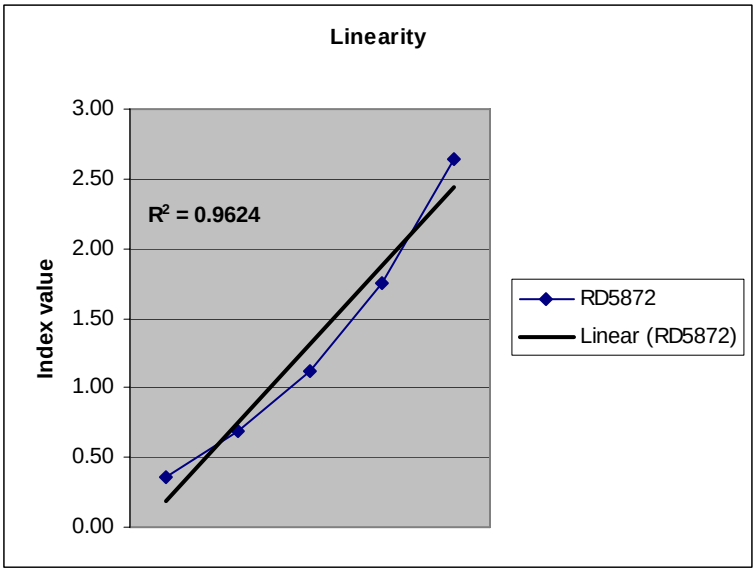
Note: all results are reported as Index Values

	Site 1			Site 2			Site 3			Inter-Assay Precison Summary		
	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Day 1	Day 2	Day 3	Site 1	Site 2	Site 3
Sample 1											3.84	3.45
mean	3.38	3.52	3.50	3.60	4.21	3.69	3.43	3.54	3.38	0.10	0.29	0.10
sd	0.10	0.06	0.08	0.03	0.12	0.06	0.08	0.10	0.07	0.03	0.07	0.03
%CV	2.8%	1.8%	2.2%	1.0%	2.9%	1.5%	2.3%	2.7%	2.1%			
Sample 2										2.90	3.02	2.97
mean	2.89	2.90	2.86	2.96	3.18	2.92	2.99	2.97	2.95	0.10	0.13	0.06
sd	0.03	0.02	0.09	0.05	0.04	0.06	0.03	0.03	0.07	0.02	0.04	0.02
%CV	1.0%	0.8%	3.0%	1.6%	1.4%	2.1%	1.1%	2.7%	2.3%			
Sample 3										0.20	0.36	0.19
mean	0.26	0.23	0.24	0.34	0.40	0.33	0.19	0.20	0.18	0.00	0.03	0.01
sd	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.07	0.10	0.07
%CV	5.0%	2.3%	3.4%	2.1%	2.9%	3.8%	3.8%	7.5%	8.0%			
Sample 4										0.10	0.14	0.11
mean	0.13	0.11	0.10	0.15	0.14	0.13	0.12	0.13	0.10	0.00	0.01	0.01
sd	0.00	0.00	0.00	0.01	0.01	0.01	0.01	0.01	0.01	0.11	0.07	0.13
%CV	3.6%	3.6%	2.6%	5.0%	4.0%	7.1%	7.5%	7.3%	8.5%			
Sample 5										0.90	0.87	0.93
mean	0.91	0.89	0.94	0.82	0.89	0.91	0.95	0.91	0.93	0.00	0.05	0.03
sd	0.01	0.02	0.02	0.02	0.01	0.02	0.02	0.03	0.02	0.03	0.05	0.03
%CV	1.5%	2.5%	2.4%	2.8%	1.5%	1.8%	2.6%	2.8%	1.7%			
Sample 6										0.90	0.84	0.95
mean	0.90	0.89	0.88	0.73	0.87	0.91	1.00	0.93	0.91	0.00	0.08	0.05
sd	0.02	0.02	0.01	0.02	0.02	0.01	0.03	0.02	0.03	0.02	0.10	0.05
%CV	3.0%	2.0%	0.9%	3.3%	2.4%	1.4%	3.3%	2.5%	3.4%			

		Between Site Summary
Sample 1	mean	3.6
	sd	0.3
	%CV	7.1%
Sample 2	mean	3.0
	sd	0.1
	%CV	3.4%
Sample 3	mean	0.3
	sd	0.1
	%CV	28.1%
Sample 4	mean	0.1
	sd	0.0
	%CV	14.1%
Sample 5	mean	0.9
	sd	0.0
	%CV	4.6%
Sample 6	mean	0.9
	sd	0.1
	%CV	8.0%

b. *Linearity/assay reportable range:*
Linearity

Four positive samples were tested neat and at two-fold serial dilutions using the Zeus Scientific VZV IgM ELISA Test System. A representative plot of test results for one of the samples demonstrates the linearity of the assay.

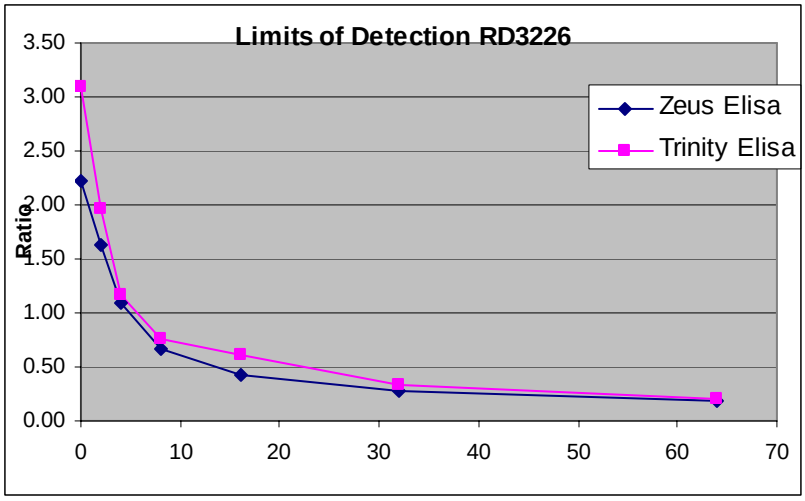


c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

Plate	18 months 2-8°C unopened
	60 days 2-8°C after opening storage envelope
Conjugate	18 months 2-8°C
Controls	18 months 2-8°C
Calibrators	18 months 2-8°C
Diluent	18 months 2-8°C
Substrate	18 months 2-8°C
Stop	18 months 2-25°C
Wash	18 months 2-8°C
	30 days 2-8°C after dilution
	7 days 20-25°C after dilution

d. *Detection limit:*
Four strongly positive samples were serially diluted and tested using the Zeus Scientific VZV IgM Test System and the predicate test system.

A representative graph of one of the samples is presented below. The results demonstrate that the Zeus Scientific VZV IgM ELISA Test System has comparable limits of detection to the predicate ELISA test system.



e. Analytical specificity:

Interfering Substances

Interfering Substances were done based on industry standard levels of test concentrations recommended in CLSI EP7-A2. The data is presented in the following table:

Interfering Substance Study

Zeus Scientific VZV IgM ELISA

	Spiked Level	SAMPLE 1 VZV IgM Positive	SAMPLE 1 % Positive Signal	SAMPLE 2 VZV IgM Borderline	SAMPLE 2 % Positive Signal	SAMPLE 3 VZV IgM Negative	SAMPLE 3 % Positive Signal
Control-PBS	N/A	3.67		0.88		0.07	
ɔntrol-Ethanol	N/A	3.59		0.82		0.07	
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		0.89		0.06	

As depicted in the table above, the positive samples showed a range of recovery from 110.66% with the high spike of hemoglobin to a low of 48.6% with the high spike of IgG. The negative sample showed a range of recovery from 400% with the high spike of IgG to a low of 88.24% with the low spike of albumin. The borderline sample showed a range of recovery of 109.64%

with the high spike of hemoglobin to a low of 83% with the low spike of IgG. Some elevation of signal in the presence of excess hemoglobin was noted. The anti-IgG absorbent (SaVE 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.

Cross-Reactivity

A minimum of 10 samples, negative for VZV IgM, were acquired and the reactivity confirmed using the predicate device. The 10 samples were subsequently tested for cross-reactivity. In all cases the specimens remained negative for VZV IgM. Please refer to the data below. All results are presented as Index Values except where noted.

Sample ID	EBV VCA IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
EBV M 5	3.54	0.77
EBV M 13	4.95	0.50
EBV M 15	1.94	0.40
EBV M 16	3.42	0.33
EBV M 17	5.23	0.52
EBV M 19	2.24	0.18
EBV M 20	1.19	0.10
431062	6.03	0.76
430410	3.50	0.32
430411	3.80	0.83

Sample ID	CMV IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
CMV M 33	5.22	0.66
CMV M 34	4.42	0.53
CMV M 35	1.63	0.64
CMV M 36	1.45	0.21
CMV M 37	1.57	0.26
CMV M 41	2.65	0.42
RD3901	6.92	0.89
00177	9.00	0.13
429023.00	6.34	0.86
429057.00	3.33	0.78

Sample ID	IU/mL RF IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
RF M 2	24.2	0.08
ARF 1	16.7	0.06
ARF 2	93.9	0.72
ARF 3	65.5	0.53
ARF 4	45.4	0.40
ARF 5	19.5	0.06
ARF 6	71.4	0.17
430066	85.8	0.66
430067	93.9	0.39
436932	25.6	0.21

Sample ID	Lyme IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
Lyme M 2	4.41	0.43
Lyme M 7	3.76	0.40
Lyme M 13	5.27	0.56
Lyme M 16	1.16	0.21
Lyme M 17	3.43	0.37
Lyme M 18	1.59	0.21
Lyme M 20	3.27	0.32
Lyme M 23	4.35	0.64
430068.00	2.26	0.64
436804.00	3.51	0.61

Sample ID	Mumps IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
Mumps1	5.40	0.17
Mumps2	5.30	0.14
Mumps3	4.80	0.16
Mumps4	4.40	0.13
Mumps5	2.18	0.09
Mumps6	1.48	0.08
Mumps7	4.88	0.11
Mumps8	4.17	0.13
Mumps9	3.65	0.11
Mumps10	2.81	0.10

Sample ID	Toxo IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
Toxo M 38	1.42	0.36
Toxo M 45	2.95	0.15
Toxo M 46	3.34	0.71
Toxo M 47	3.18	0.41
SX36034	1.86	0.23
RD4024	1.64	0.79
430472	1.95	0.18
434830	1.99	0.25
434831	2.06	0.21
434832	2.11	0.26

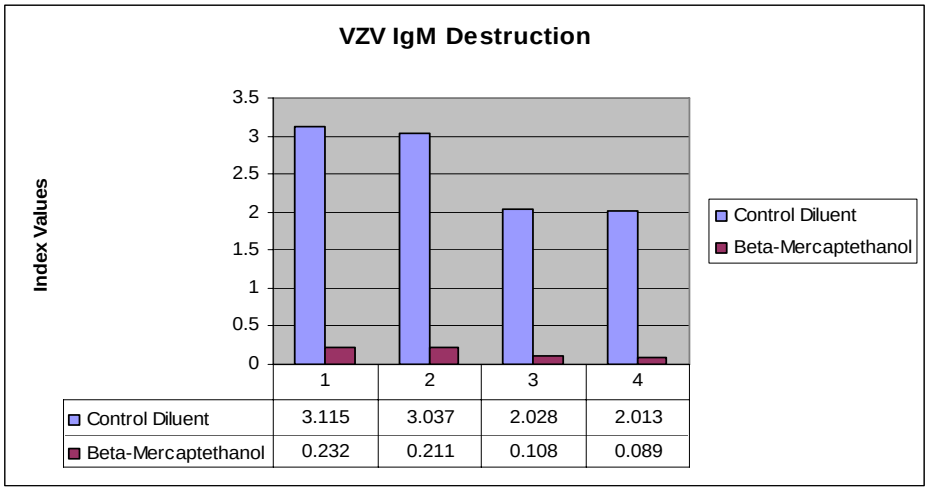
Sample ID	Measles IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
Measles1	2.49	0.31
Measles2	1.61	0.26
Measles3	1.53	0.23
Measles4	1.82	0.26
Measles5	1.32	0.24
Measles6	2.21	0.30
Measles7	1.64	0.24
Measles8	1.25	0.20
Measles9	2.14	0.30
Measles10	2.68	0.30

Sample ID	Rubella IgM ELISA Result	Zeus Scientific VZV IgM ELISA Result
RM 18	1.15	0.20
RM 19	1.51	0.33
RM 20	1.96	0.28
RM 35	1.93	0.19
RM 36	1.47	0.04
RM 37	2.55	0.08
RD3847	2.29	0.63
RD4814	2.91	0.18
437706	2.09	0.18
RD6766	2.24	0.10

Result Key:
Positive
Equivocal
Negative

IgM Destruction

A VZV IgM destruction experiment was performed to assure that the antibody which is detected by the Zeus Scientific VZV IgM ELISA Test System is indeed IgM antibody. 2% Mercaptoethanol was the IgM destroying agent used in this study. Results of the IgM destruction experiment are depicted below:



The results of the VZV IgM destruction study clearly demonstrate that the IgM antibody was destroyed, capturing the information that the antibody detected by the Zeus Scientific VZV IgM ELISA Test System is indeed VZV IgM antibody.

IgG/RF Effective Removal

The Zeus Scientific VZV IgM ELISA Test System provides sample diluent which binds IgG and Rheumatoid factor that could potentially cross-react with immobilized IgM antigen during the assay procedure. The effective elimination of IgG and rheumatoid factor reactivity in the VZV IgM test system and specific IgM reactivity is demonstrated in the following table:

	IgG Sample Diluent				IgM Sample Diluent			
	IgG Conj		IgM Conj		IgG Conj		IgM Conj	
Sample ID	OD	ISR	OD	ISR	OD	ISR	OD	ISR
VZG+ 7	2.902	8.221	0.189	0.536	0.002	0.006	0.11	0.311
VZG+ 19	>3.0	8.497	0.23	0.651	0.0	0.0	0.068	0.192
RF+ 5	0.13	0.369	0.016	0.045	0.006	0.016	0.017	0.047
VZM+ RD5161	2.914	8.254	0.776	2.197	0.0	0.001	0.728	2.063
VZM- 426642	>3.0	8.497	0.076	0.214	0.0	0.0	0.023	0.006
VZM- 418523	1.335	3.78	0.172	0.487	0.001	0.003	0.099	0.282
VZG7/RF5	2.325	6.586	0.255	0.721	0.0	0.0	0.08	0.227
VZG19/RF5	2.768	7.84	0.213	0.604	0.0	0.0	0.044	0.124

f. Assay cut-off:

Establishment and Verification of Cut-off

The cut-off corresponds roughly to the mean plus (X) times the Standard Deviation of a negative population, X being the multiplication factor necessary to optimize the assay results. 25 known negative samples, confirmed by the predicate device were assayed to establish the cut-off. Additionally, a minimum of 5 known positive samples, also confirmed by the predicate device were tested. The results of the known positive samples were ascertained to exceed the theoretical cut-off as well as the negative samples were ascertained to fall below the theoretical cut-off.

	Zeus Scientific, Inc. VZV IgM ELISA			
	Run 1	Run 2	Run 3	Trinity
Sample	Ratio	Ratio	Ratio	Biotech
EN1	0.126	0.097	0.147	0.030
EN2	0.278	0.219	0.251	0.120
EN3	0.115	0.099	0.105	0.060
EN4	0.229	0.198	0.275	0.080
EN5	0.872	0.743	0.862	0.140
EN6	0.226	0.189	0.230	0.070
EN7	0.064	0.037	0.059	0.020
EN8	0.208	0.141	0.180	0.060
EN9	0.224	0.189	0.226	0.120
EN10	0.150	0.124	0.159	0.090
EN11	0.369	0.322	0.374	0.100
EN12	0.257	0.213	0.271	0.050
EN13	0.206	0.166	0.207	0.120
EN14	0.213	0.186	0.225	0.060
EN15	0.241	0.172	0.236	0.090
EN16	0.352	0.305	0.329	0.130
EN17	0.083	0.068	0.093	0.020
EN18	0.262	0.220	0.279	0.090
EN19	0.200	0.182	0.223	0.170
EN20	0.187	0.176	0.227	0.170
EN21	0.178	0.152	0.193	0.060
EN22	0.175	0.141	0.170	0.030
EN23	0.303	0.257	0.310	0.120
EN24	0.139	0.111	0.134	0.030
EN25	0.366	0.285	0.348	0.130
RD5872	3.826	3.605	3.876	3.380
RD5161	2.005	1.962	2.053	3.090
RD3226	2.250	2.340	2.506	3.780
RD3237	2.993	2.890	3.039	4.380
BM120692	3.453	3.333	3.474	3.750
BM121251	2.773	2.630	2.806	2.900
BM124469	3.360	3.250	3.199	3.220

Mean =	0.241	0.200	0.245
Std Dev =	0.154	0.133	0.151
6X Std Dev =	0.924	0.797	0.904
Mean + 6X Std Dev =	1.165	0.996	1.148
Average	1.165	0.996	1.148

The average equals the established cut-off for the Zeus Scientific VZV IgM ELISA Test System which is 1.1.

All 25 known negative samples fall below the established cut-off, as well as all the known positive samples tested in triplicate, results follow, exceed the established cut-off.

2. Comparison studies:

   a. Method comparison with predicate device:

   See linearity section M.1.b, detection limit section M.1.d and cutoff section M.1.f.

   b. Matrix comparison:

   Not applicable

3. Clinical studies:

   a. Clinical Sensitivity:

   Not applicable

   b. Clinical specificity:

   Not applicable

   c. Other clinical supportive data (when a. and b. are not applicable):

   A comparative study was performed to demonstrate the equivalence of the Zeus Scientific VZV IgM ELISA test system to another VZV IgM ELISA test system currently in commercial distribution. The performance of the Zeus Scientific VZV IgM ELISA test system was evaluated in a three-site clinical investigation. Briefly, there was a total of 338 samples tested: 131 at site one, 53 at site 2 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 6 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 both prospective and retrospective samples:

Prospective Samples:Combined Sites

Commercial ELISA Results

Zeus Scientific,Inc	+	-	+/-	Totals
+	6	4	2	12
-		281		281
+/-		7	2	9
Totals	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** = 92.6% to 97.6%

Prospective and Retrospective Samples:Combined Sites

Commercial ELISA Results

Zeus Scientific,Inc	+	-	+/-	Totals
+	38	4	4	46
-		282		282
+/-	1	7	2	10
Totals	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

NOTE: The test is for *in vitro* use only. The performance of this assay has not been established for neonates, immunocompromised populations, cord blood or pre-transplant patients. The use of whole blood or plasma is not established.

4. Clinical cut-off**:**

   See cut-off section M.1.f.

5. Expected values/Reference range:

   See linear range section M.1.b and LOD section M.1.d.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.