510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K103363

B. Purpose for Submission: Clearance of New Device

C. Measurand: Herpes Simplex Virus (HSV-1) type specific IgG antibodies to the HSV glycoprotein G (gG) 1 recombinant antigen.

D. Type of Test: Enzyme-linked Immunosorbent Assay (ELISA)

E. Applicant: Zeus Scientific, Inc.

F. Proprietary and Established Names: ZEUS ELISA HSV gG-1 IgG Test System

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3305. Herpes Simplex Virus Serological Reagents
2. Classification: Class II
3. Product code: MXJ, Enzyme linked immunosorbent assay, Herpes Simplex Virus, HSV-1
4. Panel: Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The ZEUS ELISA HSV gG-1 IgG Test System is intended for the qualitative detection of type specific IgG class antibodies to Herpes Simplex Virus Type 1 (HSV-1) in human serum. The test is intended for testing sexually active individuals or pregnant women for aiding in the presumptive diagnosis of HSV-1 infection.
   The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The test is not intended for donor screening or for self testing.
   The performance of this assay has not been established for use in a pediatric population, neonates, or immunocompromised patients.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Spectrophotometer

I. Device Description:

The ZEUS ELISA HSV gG-1 IgG Test System is an immunoassay for the qualitative detection of IgG antibodies to HSV glycoprotein G (gG) 1 in human serum. The test system consists of recombinant HSV-1 antigen and Horse Radish Peroxidase Conjugated goat anti-human IgG (Fc chain specific) to detect IgG class antibodies to HSV-1 in human sera.

J. Substantial Equivalence Information:

1. Predicate device name(s): Reference Method for clinical evaluation

   HerpeSelect® 1 and 2 Immunoblot IgG (Focus Diagnostics)

2. Predicate Numbers (s): K000238

Comparison with predicate:

Similarities		
Item	**ZEUS ELISA HSV gG-1 IgG**	**Focus HerpeSelect Immunoblot IgG**
Intended Use	The ZEUS ELISA HSV gG-1 IgG Test System is intended for the qualitative detection of type specific IgG class antibodies to HSV-1 in human serum. In conjunction with the ZEUS ELISA HSV gG-2 IgG Test System, the test is intended for testing sexually active individuals or pregnant women for aiding in the presumptive diagnosis of HSV infection. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The test is not intended for donor screening or for self testing. The performance of this assay has not been established for use in a pediatric population, neonates or immunocompromised patients.	Focus Diagnostics' HerpeSelect 1 and 2 Immunoblot IgG test is intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-1 and HSV-2 in human sera. The test is indicated for testing sexually active adults or pregnant women for aiding in the presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection. The performance of this assay has not been established for use in a pediatric population, for neonatal screening, for testing of immunocompromised patients, for use by a point of care facility or for use with automated equipment.
Assay	Immunoassay	Immunoassay

Sample Matrix	Human Serum	Human Serum
Analyte	Human IgG	Human IgG
Antigen Used	Recombinant HSV gG-1 antigen	Recombinant HSV gG-1 antigen
Conjugate	Goat anti-human IgG; Fc chain specific	Goat anti-human IgG; Fc chain specific
Controls	2 (Negative and Positive)	2 (Negative and Positive)
Calibrators	Includes Calibrator (human serum)	Cutoff/Positive control
Differences		
Item	**ZEUS ELISA HSV gG-1 IgG**	**Focus HerpeSelect Immunoblot IgG**
Detection Method	Colorimetric	Visual
Solid Phase	Polystyrene 96 well plate	Nitrocellulose membrane
Substrate	TMB	Bromo-chloro-indolyl phosphate and nitroblur tetrazolium/A
Conjugate Label	Horseradish peroxidase	Alkaline Phosphatase
Interpretation Criteria	Negative is <= 0.90, Positive is >= 1.10 and Equivocal is 0.90 - 1.09	If patient band equal or darker than control band, result is positive. If band is lighter than control band, result is negative
Calibrators	Includes Calibrator (human serum)	Cutoff/Positive control
Reading	Read the color change (optical density) of the wells	Visually compare each band on a strip relative to the control band four bands per strip

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

1. Class II Special Controls Guidance Document: Herpes Simplex Virus Types 1 & 2 Serological Assays, September 28, 2010. http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm227411.htm

2. CLSI EP5: Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition, Villanova PA

3. CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2nd Ed. (2005).

L. Test Principle:

The ZEUS ELISA HSV gG-1 test system is designed to detect IgG class antibodies to HSV-1 in human sera. Wells of plastic microwell strips are sensitized by passive adsorption with recombinant HSV-1 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 (Fc chain specific) 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 non-reactive 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 will be greater than the cutoff value if anti-HSV 1 IgG is present in the serum being tested.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Precision was evaluated internally at Zeus Scientific. The study was conducted as follows: twelve samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the ZEUS ELISA HSV gG-1 kit. Two samples each were selected that were negative, high negative, near cut-off, low positive, moderate positive and high positive. On each day of testing, the samples were aliquoted in duplicate and tested. This was repeated in a second run on the same day by a different technologist for a total of twenty days (2 replicates x 2 runs/day x 20 days = 80 replicates per sample). The precision data were analyzed according to the principles described in the Clinical Laboratory Standards Institute guidance EP5-A2, revised November 2004. The standard deviation (SD) and percent coefficient of variation (%CV) were calculated. Results are shown in Table 1.

Table 1. Summary of In-house Precision HSV gG-1 IgG

Panel	Sample	Mean	Within-Run		Within -Day		Between-Run		Total	
Member	N	Index Value	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive	80	6.369	0.44	6.80%	0.44	6.80%	0.2	3.20%	0.51	7.90%
High Positive	80	4.13	0.12	2.90%	0.21	5.10%	0.2	4.70%	0.28	6.70%
Mod Positive	80	2.344	0.07	2.90%	0.1	4.20%	0.08	3.40%	0.15	6.30%
Mod Positive	80	2.254	0.06	2.60%	0.1	4.20%	0.08	3.60%	0.15	6.50%
Low Positive	80	1.293	0.04	3.30%	0.07	5.40%	0.06	4.80%	0.09	7.00%
Low Positive	80	1.425	0.06	4.40%	0.1	6.70%	0.08	5.30%	0.12	8.30%
Near Cut-off	80	0.993	0.05	4.70%	0.07	7.20%	0.06	6.50%	0.08	8.20%
Near Cut-off	80	0.978	0.03	3.30%	0.07	7.00%	0.07	7.00%	0.08	8.70%
High Negative	80	0.763	0.04	5.40%	0.06	8.30%	0.06	7.40%	0.08	10.30%
High	80	0.764	0.03	3.90%	0.07	8.60%	0.07	8.60%	0.08	11.00%

Negative										
Negative	80	0.097	0.01	6.50%	0.01	15.20%	0.01	15.40%	0.02	24.70%
Negative	80	0.096	0.01	7.40%	0.01	15.60%	0.01	15.90%	0.02	23.00%
Non-Reactive Control	80	0.125	0.01	8.60%	0.02	13.00%	0.01	11.90%	0.03	20.50%
Reactive Control 1	80	9.064	0.37	4.10%	0.43	4.80%	0.3	3.30%	0.53	5.80%

Reproducibility was evaluated internally and at two external clinical sites. The study was conducted as follows: twelve samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the ZEUS ELISA HSV gG-1 IgG test system. Two samples each were selected that were negative, high negative, near cut-off, low positive, moderate positive and high positive. To assess reproducibility, on each day of testing, each sample was aliquoted in duplicate, each aliquot was tested in triplicate in two runs by two operators resulting in twelve results per day. The samples were tested for five days at three sites. This was repeated in a second run by a second technologist resulting in twelve results per day. This was repeated for five days at each site and the resulting data used for analysis (3 replicates x 4 runs/day x 5 days x 3 sites = 180 replicates per sample). Results are shown in Table 2.

Table 2. Summary of Reproducibility HSV gG-1 IgG

Panel	Sample	Mean	Within-Run		Within -Day		Between-Run		Between-Site		Total	
Member	N	Index Value	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
High Positive	180	3.852	0.25	6.40%	0.26	6.70%	0.1	2.70%	0.26	6.80%	0.26	6.80%
High Positive	180	6.022	0.46	7.70%	0.51	8.50%	0.27	4.50%	0.52	8.70%	0.53	8.80%
Mod Positive	180	2.311	0.15	6.60%	0.18	7.80%	0.11	4.80%	0.19	8.10%	0.19	8.10%
Mod Positive	180	2.209	0.12	5.60%	0.15	6.80%	0.09	4.10%	0.16	7.30%	0.17	7.60%
Low Positive	180	1.237	0.09	6.90%	0.09	7.70%	0.05	4.10%	0.1	8.40%	0.1	8.40%
Low Positive	180	1.285	0.09	7.20%	0.11	8.70%	0.06	4.90%	0.12	9.00%	0.12	9.10%
Near Cut-off	180	0.938	0.07	7.00%	0.08	8.20%	0.05	5.10%	0.08	8.70%	0.08	8.70%
Near Cut-off	180	0.93	0.07	7.20%	0.08	8.80%	0.06	6.40%	0.09	9.40%	0.09	9.90%
High Negative	180	0.683	0.05	7.90%	0.07	9.90%	0.04	6.40%	0.07	10.30%	0.07	10.50%
High Negative	180	0.732	0.06	8.00%	0.07	9.20%	0.04	5.00%	0.07	9.40%	0.08	10.50%
Negative	180	0.076	0.01	19.50%	0.02	23.90%	0.01	14.40%	0.02	25.30%	0.02	27.40%
Negative	180	0.079	0.02	21.30%	0.03	26.80%	0.01	16.50%	0.02	28.60%	0.02	30.50%
Non-Reactive Control	180	0.109	0.02	16.20%	0.02	19.20%	0.01	12.70%	0.02	21.70%	0.03	27.10%
Reactive Control	180	9.011	0.53	5.90%	0.56	6.30%	0.2	2.20%	0.6	6.60%	0.65	7.20%

b. *Linearity/assay reportable range:*
N/A

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no standard available for measuring HSV antibody in serum

d. *Detection limit:*
Not applicable. This device is for qualitative HSV-1 antibody detection.

e. *Analytical specificity:*

Cross Reactivity: A study was conducted to assess cross reactivity with the ZEUS ELISA HSV gG-1 IgG test system using sera that were sero-positive to EBV VCA IgG, ANA, Rubella, VZV IgG, CMV, Measles, *Treponema pallidum*, Gonorrhea, HPV, Chlamydia, rheumatoid factor (RF), *Toxoplasma gondii*, and HSV IgG-2. Since the antigen used in the Zeus ELISA HSV gG-1 IgG Test System is purified from a yeast cell lysate (*Saccharomyces cerevisiae*) containing recombinant IgG1 glycoprotein antigen specific to HSV-1, potential cross reactivity with sero-positive specimens against *Saccharomyces cerevisiae* was also assessed. Micro-particle and ELISA immunoassay test systems manufactured by various companies for commercial distribution were used to determine the sero-positivity of the samples. Ten samples for each possible cross-reactant were tested. In total, 140 samples were tested for possible cross reactivity with 14 analytes. None of the samples showed cross-reactivity with any of the analytes tested. The results of this study are summarized in Table 3.

Table 3. Cross Reactivity Summary

HSV gG-1 Cross reactivity Study	
Analyte	positive/tested
EBV VCA IgG	0/10
ANA	0/10
Measles	0/10
Rubella	0/10
CMV	0/10
VZV	0/10
T. pallidum	0/10
Gonorrhea	0/8
HPV	0/9
Chlamydia	0/10
RF	0/10
T. gondii	0/10
S. cerevisiae	0/10
HSV gG-2	0/10

Specimens known to contain potentially cross reactive antibodies to *Candida albicans* have not been tested with this device, therefore it is unknown if there is any cross reactivity with these antibodies. This is indicated in the limitation section of the package insert.

Interfering Substances: The effect of potentially interfering substances on sample results generated using the ZEUS ELISA HSV gG-1 IgG test system was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids.

The level of each potentially interfering substance is as follows:
Bilirubin: 1mg/dL (low), 15 mg/dL (high)
Albumin: 3.5 g/dL (low), 5 g/dL (high)
Cholesterol: 150 mg/dL (low), 250 mg/dL (high)
Triglycerides: 150 mg/dL (low), 500 mg/dL (high)
Hemoglobin: 10 g/dL (low), 20 g/dL (high)
Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples for HSV gG-1 IgG were chosen based on their performance on the investigational device: positive, borderline and negative. The potentially interfering substances were added to the samples. Test and control samples were evaluated in replicates of ten. All positive and borderline samples showed a change of signal less than 20%. All positive samples remained positive. Borderline samples did remain within 20% of the control result and were still in the borderline range. No sample went from positive to negative or negative to positive. The negative sample showed a change of signal (>20%) with the high spike of albumin (167.9%), the low and high spikes of hemoglobin (205.4 and 139.3% respectively), intralipid (64.3 and 62.5% respectively), cholesterol (168.8 and 75.0% respectively) and the low spike of triglycerides (126.3%). The negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

f. *Assay cut-off:*

The cut off for this assay was established using 25 negative control specimens as well as 9 clinically characterized specimens for each antigen. The mean and standard deviation were established for the negative population. Using a mathematical calculation involving this data, a theoretical cut-off was established and validated with the characterized specimens. Based upon the results of this testing, the manufacturer established the following guidelines for interpretation of patient samples.

Interpretations:
Index Values or OD ratios are interpreted as follows:

Result	Index Value or OD Ratio
Negative	**≤ 0.90**
Equivocal	**0.91 to 1.09**
Positive	**≥ 1.10**

1. An OD ratio ≤ 0.90 indicates no detectable IgG antibody to HSV-1.
2. Specimens with OD ratio values in the equivocal range (0.91 – 1.09) should be retested in duplicate. If on re-testing one of the two samples remains equivocal, the samples should be tested by an alternate serological procedure such as Westen Blot or re-evaluated by drawing another sample one to three weeks later.
3. An OD ratio ≥ 1.10 indicates that HSV-1 IgG antibodies were detected.
4. The numeric value of the final result above the cutoff is not indicative of the amount of anti-HSV 1 IgG antibody present.
5. Test results should be interpreted in conjunction with the clinical history, epidemiological data and other information available to the attending physician in evaluating the patient.
6. False positive test results may occur. Repeat testing or testing with a different device may be indicated in some settings e.g., patients with low likelihood of HSV infection.

2. Comparison studies:

a. Method comparison with predicate device: Comparative studies were performed using a total of 788 samples at three clinical sites to demonstrate the equivalence of the ZEUS ELISA HSV gG-1 test system to the reference method (a commercially distributed HSV 1 and 2 immunoblot test system). Five hundred and eighty-eight samples were from the Intended Use populations of sexually active individuals (n=336) and pregnant women (n=252) with an HSV test requested. One hundred additional samples from a low prevalence population of 17-19 year old were tested as were 100 samples acquired from the CDC (CDC panel). The clinical sites and quantity of samples tested are summarized in Table 4.

Table 4. Samples Tested at the Clinical Sites

	Number of Samples Tested at Each Site			
Populations	Site 1	Site 2	Site 3	Total
Sexually Active Individuals	100	136	100	336
Pregnant women	33	155	64	252
Low Prevalence Population	33	33	34	100
CDC Panel	33	25	42	100

Results of this comparative study in the three sites combined are presented below. The data was analyzed counting any discordant equivocal results between the comparator and the investigational device against the performance of the investigational device.

Performance in the Intended Use Population of Sexually Active Individuals: A total of 336 prospective, unselected samples from sexually active individuals with an HSV-1 test ordered were tested with the ZEUS ELISA HSV gG-1 IgG Test System and compared with a commercially available immunoblot test. The samples were submitted for HSV-1 antibody testing, sequentially numbered, de-identified and archived. Results are presented in Table 5.

Table 5. Sexually Active Individuals

		Immunoblot						
		Positive	**Equivocal**	**Negative**	**Site Total**		**% Agreement***	**95% CI**
ZEUS ELISA HSV gG-1	**Positive**	201	2	9	212	PPA	99.5% (201/202)	97.3-100%
	Equivocal	0	1	0	1			
	Negative	0	1	122	123	NPA	91.7% (122/133)	85.7-95.8%
	Site Total	201	4	131	336			

*Equivocal results in one test but not the other were treated as discrepant and included in the calculations.

Performance in the Intended Use Population of Pregnant Women: A total of 252 prospective, unselected samples from pregnant women with an HSV-1 test ordered were tested with the ZEUS ELISA HSV gG-1 IgG Test System and compared with a commercially available immunoblot test. One hundred and twenty-one samples were from women in the first trimester, 64 from women in the second trimester and 67 from women in the third trimester. The samples were submitted for HSV-1 antibody testing, sequentially numbered, de-identified and archived. Results are presented in Table 6.

Table 6. Pregnant Women

		Immunoblot						
		Positive	**Equivocal**	**Negative**	**Site Total**		**% Agreement***	**95% CI**
ZEUS ELISA HSV gG-1	**Positive**	175	0	3	178	PPA	99.4% (175/176)	96.9-100%
	Equivocal	0	0	5	5			
	Negative	1	0	68	69	NPA	89.5% (68/76)	80.3-95.3%
	Site Total	176	0	76	252			

**Equivocal results in one test but not the other were treated as discrepant and included in the calculations.

Performance in a Low Prevalence Population: A total of 100 samples requested to be collected from 17-19 year old in a non-STD setting. These samples were purchased from a vendor. Results are presented in Table 7.

		Table 7. Low Prevalence Population						
		Immunoblot						
		Positive	Equivocal	Negative	Site Total		% Agreement*	95% CI
ZEUS ELISA HSV gG-1								
	Positive	27	0	3	30	PPA	100.0% (27/27)	89.5-100%
	Equivocal	0	0	2	2			
	Negative	0	0	68	68	NPA	93.2% (68/73)	84.7-97.7%
	Site Total	27	0	73	100			

*Equivocal results in one test but not the other were treated as discrepant and included in the NPA calculations.

CDC HSV-1 IgG Panel: A total of 100 samples were obtained from the CDC for analysis. The performance of the ZEUS ELISA HSV gG-1 IgG Test System was assessed using a masked, well characterized HSV serum panel from the CDC. The panel consists of 50 HSV-1 IgG positive samples and 50 HSV-1 IgG negative samples. Twenty-four of the 50 HSV-1 IgG positive samples were positive for bothHSV-1 IgG and HSV-2 IgG. The results are presented in Table 8 to convey further information on the performance of the test kit and do not imply endorsement of the assay by the CDC.

		Table 8. CDC HSV Panel						
		CDC HSV-1 Results						
		Positive	Equivocal	Negative	Site Total		% Agreement*	95% CI
ZEUS ELISA HSV gG-1								
	Positive	50	1	1	52	PPA	100.0% (50/50)	94.2-100%
	Equivocal	0	0	0	0			
	Negative	0	0	48	48	NPA	96.9% (48/50)	86.3-99.5%
	Site Total	50	1	49	100			

*Equivocal results in one test but not the other were treated as discrepant and included in the NPA calculations.

b. *Matrix Comparison :* N/A

3. Clinical studies:

a. Clinical Sensitivity: N/A

b. Clinical specificity: N/A

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

4. Clinical cut-off**:** N/A

5. Expected values/Reference range:

The observed prevalence with the Intended Use populations was assessed internally and externally at two sites with unselected, masked and archived sera. With the sexually active individuals (n=336) the observed prevalence with the ZEUS ELISA HSV gG-1 was 63.7% (214/336).
In the population of pregnant women (n=252), the observed prevalence with the ZEUS ELISA HSV gG-1 was 70.5% (177/251). One sample was excluded from this analysis due to unknown age.
The following two tables (Table 9 and Table 10) summarize the prevalence observed when the investigational device was tested on the two populations (sexually active individuals and pregnant women). The observed prevalence with the investigational device in each age group tested in the two populations are summarized in tables 9 and 10.

Table 9. Observed Prevalence with Sexually Active Individuals

		HSV gG-1				Observed % Prevalence
Age	Sex	Positive	Equivocal	Negative	total	HSV gG-1
15-19	Male	4		7	11	36.4
	Female	11		11	22	50.0
20-29	Male	13	1	20	34	38.2
	Female	62		29	91	68.1
30-39	Male	16		9	25	64.0
	Female	42		18	60	70.0
40-49	Male	17		7	24	70.8
	Female	17		4	21	80.9
50-59	Male	16		11	27	59.3
	Female	9		1	10	90.0
60-69	Male	6		3	9	66.7
	Female			1	1	0.0
70 +	Male	1			1	100
	Female				0	0.0
Sub-total	Male	73	1	57	131	55.7
	Female	141	0	64	205	68.8
	Total	214	1	121	336	63.7%

Table 10. Observed Prevalence with Pregnant women

	HSV gG-1				Observed % Prevalence
Age	Positive		Negative	total	HSV gG-1
15-19	16		8	24	66.7
20-29	101	3	45	149	67.8
30-39	48	2	13	63	76.2
40-49	12		3	15	80.0
Total	177	5	69	251	70.5%

one sample submitted with age unknown was excluded

The hypothetical predictive values for the two populations are shown in the table below. The calculations are based on the ZEUS ELISA HSV gG-1 IgG having

Table 11. Prevalence vs. Hypothetical Predictive Values

	HSV gG-1 IgG			
	Sexually Active Individuals		Pregnant Women	
Prevalence	PPV	NPV	PPV	NPV
50%	92.30%	99.50%	90.40%	99.30%
40%	88.90%	99.60%	86.30%	99.60%
30%	83.70%	99.80%	80.20%	99.70%
25%	80.00%	99.80%	75.90%	99.80%
20%	75.00%	99.90%	70.30%	99.80%
15%	67.90%	99.90%	62.60%	99.90%
10%	57.10%	99.90%	51.30%	99.90%
5%	38.70%	100.00%	33.30%	100.00%

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.