510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K072178

B. Purpose for Submission:

New device.

C. Measurand:

Herpes Simplex Virus (HSV-1 and HSV-2) type specific IgG antibodies to the HSV glycoprotein G (gG) 1 antigen and gG2 antigen.

D. Type of Test:

Multiplexed micro particle immunoassay based on Luminex technology

E. Applicant:

Zeus Scientific, Inc.

F. Proprietary and Established Names:

AtheNA Multi-Lyte HSV 1 & 2 IgG Test System

G. Regulatory Information:

1. Regulation section:

   21 CFR 866.3305 - Herpes Simplex Virus Serological Reagents

2. Classification:

   Class II (Special Controls)

3. Product code:

   MXJ and MYF

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

The Zeus Scientific, Inc. AtheNA Multi-Lyte® HSV 1 & 2 IgG Test System is intended for the qualitative detection of the presence or absence of IgG antibodies to HSV-1 and HSV-2 in human serum. The test is indicated for sexually active adults and expectant mothers, as an aid for presumptively diagnosing Herpes Simplex 1 and Herpes Simplex 2.

The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2. 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, immunocompromised patients, for use by point of care facilities or for use with automated equipment.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   AtheNA Multi-Lyte® System

I. Device Description:

The AtheNA Multi-Lyte® HSV 1 & 2 IgG Test System is a multiplexed micro particle bead based immunoassay for the qualitative detection of IgG antibodies to HSV glycoprotein G (gG) 1 and 2 in human serum using the Luminex flow cytometry technology

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Reference Method for clinical evaluation: HerpeSelect® 1 and 2 immunoblot,

2. Predicate K number(s):

   K000238

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Zeus Scientific, Inc. AtheNA Multi-Lyte® HSV 1 & 2 IgG Test System is intended for the qualitative detection of the presence or absence of IgG antibodies to HSV-1 and HSV-2 in human serum. The test is indicated for sexually active adults and expectant mothers, as an aid for presumptively diagnosing Herpes Simplex 1 and Herpes Simplex 2. The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2. 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, immunocompromised patients, for use by point of care facilities or for use with automated equipment.	same
matrix	serum	same
antigen	1. Recombinant gG1 antigen (molecular weight 55 KD) 2. Recombinant gG2 antigen (molecular weight 31 KD)	1. HSV native virus antigens 2. Recombinant gG1 antigen 35-45 KD 3. Recombinant gG2 antigen 80-110 KD

Differences		
Item	Device	Predicate
Method	Multiplexed microparticle flow cytometry immunoassay	Immunoblot assay

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

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

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

L. Test Principle:

The test procedure involves two incubation steps:

1. Patient sera are diluted and the diluted test sera are incubated in a vessel containing a multiplexed bead suspension consisting of a mixture of distinguishable sets of polystyrene microspheres. Conjugated to the primary set of microspheres are HSV gG-1 and HSV gG-2 antigens. The bead mix also contains one bead set designed to detect non-specific antibodies in the patient sample (if present) and four separate bead sets used for assay calibration. If present in patient sera, HSV 1 and HSV 2 antibodies will bind to the corresponding immobilized antigen bead set. The microspheres are rinsed to remove non-reactive serum proteins.

2. Phycoerythrin-conjugated goat anti-human IgG (Fc specific) is added to the vessel and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The bead suspension is then analyzed by the AtheNA Multi-Lyte® instrument. The bead set(s) are sorted (identified) and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the Intra-Well Calibration Technology®, internal calibration bead sets are used to establish the assay's cutoff. Raw fluorescence from each distinct HSV gG-1 and HSV gG-2 antigen bead type is measured and compared against the cut-off calibrator.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

   a. *Precision/Reproducibility:*

   Assay precision was evaluated at three sites (one internal and 2 external) for three days as follows: Six samples were identified for use in the study based upon their activity on the AtheNA Multi-Lyte assay. Two samples were clearly negative, two were clearly positive and two samples were near the assay cut off. One run was performed each day at each lab, the samples were diluted twice and each dilution was run in quadruplicate, resulting in eight results per assay and 24 replicates per specimen. The study for HSV 1 and 2 is summarized below:

HSV 1

Sample ID	Within lab			Between lab		
	Index mean	Intra-assay % CV	Inter-assay % CV	Index mean	% CV of lab means	
1	26.3	15.0	18.2	26.3	21.8	
2	8.4	39.2	44.0	8.4	58.2	
3	144.8	11.9	15.9	144.8	16.6	
4	195	11.0	12.3	195	15.9	
5	311.8	8.4	9.9	311.8	10.7	
6	392.2	8.5	9.1	392.2	12.8	

HSV 2

Sample ID	Within lab			Between lab		
	Index mean	Intra-assay % CV	Inter-assay % CV	Index mean	% CV of lab means	
1	16.4	36.4	36.8	16.4	44.0	
2	20.8	27.9	31.0	20.8	40.0	
3	155.7	15.5	21.0	155.7	23.6	
4	114.2	10.6	13.7	114.2	18.1	
5	442.3	9.2	12.4	442.3	13.9	
6	356.2	8.0	14.1	356.2	16.1	

Assay repeatability was evaluated at the manufacturer site in accordance with CLSI EP5: Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition, Villanova PA. A panel of six samples (as described above) was diluted twice and tested in X replicates per day. The samples were tested on two runs per day by a different technologist for a total of twelve days. This study is summarized below:

Sample ID	HSV 1			HSV2		
	Index mean	Intra-assay % CV	Inter-assay % CV	Index mean	Intra-assay % CV	Inter-assay % CV
1	24.6	15.0	16.8	17.4	28.3	35.8
2	8.5	38.3	41.6	22.4	20.9	28.1
3	115.8	6.3	10.0	124	9.7	15.0
4	163.5	10.5	12.0	93.6	11.1	12.3
5	299.7	9.1	11.6	362	10.5	12.3
6	362.8	8.6	12.5	301.1	9.7	14.1

b. *Linearity/assay reportable range:*

Not Applicable

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

Not Applicable

d. *Detection limit:*

Not Applicable

e. *Analytical specificity:*

Potential cross-reactivity was evaluated as follows: 10 samples, dual negative for HSV IgG 1 and 2, that were sero-positive, to Measles, Mumps, EBV VCA, EBNA, Rubella, VZV, ANA, CMV by commercially available test systems manufactured by Zeus Scientific, Inc. for commercial distribution. In all cases the specimens remained negative for HSV 1 and 2 IgG. Potential cross reactivity with *E. coli* which is the recombinant vector for the gG1 and gG2 antigens used in the assay was not assessed, due to difficulties in obtaining the appropriate samples.

The effect of potential interfering substances on sample results generated using the AtheNA Multi-Lyte HSV 1 & 2 IgG test system was evaluated with the following possible interfering substances based on the guidelines established in CLSI EP7-A2 (18): albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. Three samples were chosen based on their performance on the AtheNA Multi-Lyte test system: positive (HSV-1, 818 AU/mL; HSV-2, 566 AU/mL), borderline (HSV-1, 152 AU/ml; HSV-2, 92 AU/mL) and negative (HSV-1, 62 AU/mL; HSV-2, 34AU/mL). The samples were exposed to the possible interfering substance, tested in duplicate and the mean established. All qualitative results remained unchanged; indicating that the interfering effect of the substances tested is minimal. However, the negative HSV-1 sample exhibited an increase in signal of 33% with the low spike of albumin and an increase in signal of 39% with the high spike of albumin. The negative HSV-2 sample showed a change in signal of 37% with the low spike of albumin and 28% with the high spike of albumin. The negative HSV-2 sample also showed changes in signal with bilirubin, 43% and 52%, albumin, 37% and 28%, hemoglobin, 53% and 52% and intralipids, 52% and 34%, low and high spikes of interfering substances respectively. The change of signal in these negative samples did not change the qualitative outcome in these samples, the results remained negative.

f. *Assay cut-off:*

The cut-off was established using 27 known negative samples, confirmed by a commercially distributed ELISA. Additionally, a minimum of 5 known positive samples, also confirmed by a commercially distributed ELISA assay 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. The cut-off was set equal to mean plus 7X SD for HSV1 and mean plus 6XSD for HSV2. The cut-off was validated by the clinical studies, the values for the mean; SD and cut-off from the clinical studies were as follows:

	HSV-1	HSV-2
Mean	15	31
SD	17	19.8
Cut-off	118	119

2. Comparison studies:

   a. *Method comparison with reference method:*

   Performance in Sexually Active Adults:

   Comparative studies were performed using a total of 317 prospectively collected samples at three clinical sites to demonstrate the equivalence of the AtheNA Multi-Lyte HSV 1 & 2 to the reference method a commercially distributed HSV 1 and 2 immunoblot test system. Zeus Scientific tested 135 samples. Two outside investigators tested 84 samples and 98 samples respectively. The samples were sequentially submitted to the laboratories, archived and masked. The samples were collected from sexually active adults between the ages of 17 and 70 and submitted for Herpes simplex antibody testing. Results of this comparative study in the three sites combined are presented below:

   The data was analyzed including counting the equivocal results from the comparator and the investigational device against the performance of the investigational device.

Table 1: HSV 1 in Sexually Active Adults

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot					
		Positive	Indeterminate	Negative	Total	Sensitivity& Specificity	95% Confidence Interval
	Positive	180	1	8	189	180/183= 98.4%	95.3-99.7
	Equivocal	0	0	1	1		
	Negative	3	0	124	133	124/134= 92.5%	86.7-96.4
	Total	183	1	133	317		

Table 2: HSV 2 in Sexually Active Adults

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot				Sensitivity& Specificity	95% Confidence Interval
		Positive	Indeterminate	Negative	Total		
	Positive	80	0	9	89	80/82= 97.6%	91.4-99.7
	Equivocal	0	0	0	0		
	Negative	1	1	226	228	226/235= 96.2%	92.9-98.2
	Total	81	1	235	317		

Performance in Pregnant Women:

Comparative studies were performed internally using masked, archived sera from 150 expectant mothers ranging in age from 18 to 48. Of these 150 expectant mothers, 50 were in their first trimester of pregnancy, 50 were in their second trimester and 50 were in their third trimester of pregnancy. The results are presented below:

Table 3: HSV 1 in Pregnant Women

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot				Sensitivity& Specificity	95% Confidence Interval
		Positive	Indeterminate	Negative	Total		
	Positive	98	0	2	100	98/98= 100%	97.0-100
	Equivocal	0	0	0	0		
	Negative	0	0	50	50	50/52= 96.2%	86.8-99.5
	Total	98	0	52	150		

Table 4: HSV 2 in Pregnant Women

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot				Sensitivity& Specificity	95% Confidence Interval
		Positive	Indeterminate	Negative	Total		
	Positive	59	0	2	61	59/59= 100%	93.9-100
	Equivocal	0	0	0	0		
	Negative	0	0	89	89	89/91= 97.8%	92.3-99.7
	Total	59	0	91	150		

Agreement with CDC Panel:

The performance of the AtheNA Multi-Lyte HSV 1 & 2 IgG assay with a masked, characterized serum panel from the CDC is presented below.

Table 5: Agreement in HSV 1 IgG antibody detection

		CDC results					
AtheNA Multi-Lyte		Positive	Negative	Total	Percent Agreement	95% Confidence Interval	
HSV 1 & 2 Test System	Positive	50	0	50	50/50= 100%	94.2-100	
	Equivocal	0	0	0			
	Negative	0	50	50	50/50= 100%	94.2-100	

Table 6: Agreement in HSV 2 IgG antibody detection

		CDC results					
AtheNA Multi-Lyte		Positive	Negative	Total	Percent Agreement	95% Confidence Interval	
HSV 1 & 2 Test System	Positive	48	1	49	48/48= 100%	94.0-100	
	Equivocal	0	0	0			
	Negative	0	51	51	51/52= 98.1%	89.7-100	
	Total	48	52	100			

Performance in a Low Prevalence Population:

The performance of the AtheNA Multi-Lyte HSV 1 & 2 test system was evaluated (internally) in a low prevalence population for genital herpes to a commercially distributed HSV immunoblot system. The low prevalence population was comprised of stored samples from 18 and 19 year old subjects previously tested for infections considered non-sexual in nature. The results of this study are summarized below:

Table 7: HSV 1 in a low Prevalence population

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot					
		Positive	Indeterminate	Negative	Total	Sensitivity& Specificity	95% Confidence Interval
	Positive	8	0	2	10	8/8= 100	63.1-100
	Equivocal	0	0	0	0		
	Negative	0	0	56	56	56/58= 96.6	88.1-99.6
	Total	8	0	58	66		

Table 8: HSV 2 in a low prevalence population

AtheNA Multi-Lyte HSV 1 & 2 Test System		HerpeSelect® 1 and 2 immunoblot					
		Positive	Indeterminate	Negative	Total	Sensitivity& Specificity	95% Confidence Interval
	Positive	3	0	1	4	3/3= 100	29.2-100
	Equivocal	0	0	1	1		
	Negative	0	0	62	62	62/63= 98.4	91.5-100
	Total	3	0	64	66		

    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):

    Not Applicable

4. Clinical cut-off:

    See 1 f

5. Expected values/Reference range:

    The observed prevalence and the hypothetical predictive values were determined

for the intended use populations (pregnant women and sexually active adults).The observed prevalence of HSV-1 and HSV- 2 in the sexually active adult population is 59.6% (HSV 1) and 23.7% (HSV 2). In the expectant mothers, the observed prevalence of HSV-1 is 66.7% and 40.7% for HSV-2. The results are summarized below:

Table 9: HSV 1 and 2 Observed prevalence in the Sexually Active Adults population

Age	Gender	HSV 1 positive	Observed HSV 1 Prevalence	HSV 2 positive	Observed HSV 2 Prevalence
17- 19	Male	4	2.1%	0	0%
	Female	7	3.7%	2	2.2%
	unknown	0	0%	0	0%
20- 29	Male	20	10.6%	6	6.7%
	Female	49	25.9%	22	24.7%
	unknown	2	1.1%	2	2.2%
30- 39	Male	16	8.5%	8	9.0%
	Female	31	16.4%	12	13.5%
	unknown	1	0.5%	1	1.1%
40- 49	Male	14	7.4%	6	6.7%
	Female	10	5.3%	7	7.9%
	unknown	0	0%	0	0%
50- 59	Male	19	10.1%	12	13.5%
	Female	6	3.2%	3	3.4%
	unknown	1	0.5%	1	1.1%
60- 69	Male	5	2.6%	4	4.5%
	Female	4	2.1%	3	3.4%
	unknown	0	0%	0	0%
Sub-total	Male	78	41.3%	36	40.4%
	Female	107	56.6%	49	55.1%
	unknown	4	2.1%	4	4.5%
Total		189/ 317	59.6%	89/ 317	28.1%

The negative and equivocal samples were removed from the table for clarity. There was one equivocal sample in the HSV 1 results and no equivocal samples in the HSV 2 results.

Table 10: HSV 1 and 2 Observed prevalence in the Expectant Mothers population.

Age	HSV 1 positive	Observed HSV 1 Prevalence	HSV 2 positive	Observed HSV 2 Prevalence
17- 19	12	12.0%	8	13.1%
20- 29	52	52.0%	37	60.7%
30- 39	25	25.0%	12	19.7%
40- 49	11	11.0%	4	6.6%
Total	100/ 150	66.7%	61/150	40.7%

Table 11: Prevalence vs. Hypothetical Predictive Values

Prevalence	Sexually active adults				Expectant Mothers			
	HSV 1		HSV 2		HSV 1		HSV 2	
	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
50%	925.3	98.3	96.2	97.6	96.3	100	97.8	100
40%	89.7	98.9	94.4	98.4	94.6	100	96.8	100
30%	84.9	99.3	91.6	98.9	91.9	100	95.1	100
25%	81.4	99.4	89.5	99.2	89.8	100	93.8	100
20%	76.6	99.6	86.5	99.4	86.8	100	91.9	100
15%	69.8	99.7	81.9	99.6	82.2	100	88.9	100
10%	59.3	99.8	74.1	99.7	74.5	100	83.5	100
5%	40.8	99.9	57.7	99.9	58.1	100	70.5	100

N. Proposed Labeling:

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

O. Conclusion:

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