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

A. 510(k) Number: K042118

B. Purpose for Submission:

Premarket clearance for a multiplex AtheNA Multi-Lyte® EBV IgG Test System incorporating EBV VCA IgG, EBNA IgG and EA IgG

C. Measurand: Antibodies to EBV VCA IgG, EBNA IgG and EA IgG

D. Type of Test:

The Zeus Scientific, Inc. AtheNA Multi-Lyte EBV test system is designed to detect IgG class antibodies in human sera to a variety of EBV antigens. Test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture of the bead suspension. The multiplexed bead suspension contains a mixture of distinguishable sets of polystyrene microspheres; three of these bead sets are conjugated with the three EBV antigens (EBV VCA, EBNA-1 and EBV EA). The bead mix also contains one bead set designed to detect non-specific binding and four separate bead sets used for assay calibration. If present in patient sera, specific antibodies will bind to the immobilized antigen on one or more of the bead sets. The microspheres are rinsed to remove non-reactive serum proteins. Using the *Intra-Well Calibration Technology*®, internal calibration bead sets are used to convert raw fluorescence into outcome (units).

E. Applicant: Zeus Scientific , Inc.

F. Proprietary and Established Names: AtheNA Multi-Lyte® Test System

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3235

2. Classification: Epstein-Barr Virus Serological Reagents

3. Product code: JRY – Antiserum, Fluorescent, Epstein-Barr Virus

4. Panel: 83

H. Intended Use:

1. Intended Use: The Zeus Scientific, Inc. AtheNA Multi-Lyte® EBV IgG Test System is intended for the qualitative detection of IgG class antibody to three separate EBV Antigens (EBV-VCA gp-125, EBV-EA "total" and recombinant EBNA-1) in human serum using the AtheNA Multi-Lyte® System. The test system is intended to be used as an aid in the laboratory diagnosis of EBV-associated infectious mononucleosis and to provide epidemiological information on the disease caused by Epstein-Barr virus.

2. Indication(s) for use: The Zeus Scientific, Inc. AtheNA Multi-Lyte EBV IgG Test System is intended for the qualitative detection of IgG class antibody to three separate EBV Antigens (EBV-VCA, EBV-EA and EBNA-1) in human serum using the AtheNA Multi-Lyte System. The test system is intended to be used with these EBV IgG markers along with anti-EBV VCA IgM to aid in the laboratory diagnosis of EBV-associated infectious mononucleosis and to provide epidemiological information on the diseases caused by EBV virus.

3. Special conditions for use statement(s): N/A

4. Special instrument requirements: This device is intended to be used with Zeus' AtheNA Multi-Lyte® System which has previously been cleared. Zeus' device is designed to be used with this device exclusively.

I. Device Description:

Assay Calibration
The AtheNA Multi-Lyte test system utilizes *Intra-Well Calibration Technology®*. *Intra-Well Calibration Technology* includes a multi-point standard curve within the bead suspension. With *Intra-Well Calibration Technology*, each well of the assay is calibrated internally without any user intervention. The standard curve is designed to self-adjust based upon the unique characteristics of the patient or control serum. Calibrator values are assigned to the internal standards by Zeus Scientific, Inc. These values are lot specific and are encoded within the lot specific Calibration CD included in the kit box.

Analyte Cut Off Values
Each analyte of the **AtheNA Multi-Lyte** test system has an assigned cut off value. Cut off values are determined by Zeus Scientific, Inc. for each kit lot, and are encoded within the lot specific Calibration CD included in the kit box.

Calculations
Through *Intra-Well Calibration Technology,* all calculations are performed automatically when using the AtheNA Multi-Lyte system. *Intra-Well Calibration Technology* performs a regression analysis of the internal standards and then adjusts the calculated unit values based upon an additional standard and the characteristics of the serum sample.

Reactive Reagents:

1 All reactive reagents contain sodium azide as a preservative at a concentration of 0.1% w/v)

2 Multiplexed bead suspension. Ready to use, 5.5 mL bottle. The suspension contains separate distinguishable 5.6 micron polystyrene beads that are conjugated with the following antigens: affinity purified EBV VCA gp125, affinity purified EA (roughly equal parts EA-D and EA-R) and recombinant EBNA-1. 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.

3 Phycoerythrin conjugated goat anti-human IgG (Fc chain specific). Ready to use, 15 mL amber bottle.

4 Human positive serum control. One, 0.2mL vial.

5 Human negative serum control. One, 0.2mL vial.

6 Sample diluent. One 50 mL bottle containing phosphate-buffered-saline. Ready to use. NOTE, the sample diluent will change color in the presence of serum.

Non-Reactive Reagents:

1 One, 96-well polystyrene assay plate

2 One, 96-well dilution plate

3   One, 96-well filtration plate for rinsing the microspheres

4   Data Labels: One label is adhered to the inside lid of the kit box
    and a second label is inside the kit box

5   Package Insert providing instructions for use

6   CD. A compact disc that includes all lot-specific kit calibration
    values required for specimen analysis and assay quality control

Materials required but not provided:

1   AtheNA Multi-Lyte System

2   Pipettes capable of accurately delivering 10 to 200 L

3   Multichannel pipette capable of accurately delivering (10-200 L)

4   Reagent reservoirs for multichannel pipettes.

5   Disposable pipette tips

6   Laboratory timer to monitor incubation steps

7   Small bath sonicator

8   Plate shaker capable of shaking at 800 RPM (optional for mixing)..

9   Vacuum aspirator and vacuum manifold for washing the microspheres

J. Substantial Equivalence Information:

1. Predicate device name(s): Diamedix EBV VCA IgG, EBNA IgG, EA IgG ELISA

2. Predicate 510(k) number(s): Diamedix EBV VCA IgG (K981812), EBNA IgG
    (K981829), and EA IgG (K981831) ELISA

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Cutoff calibrators	Cut off values are determined by Zeus Scientific, Inc. for each kit lot, and are encoded within the lot specific Calibration CD included in the kit box	Cutoff value is determined with a factory determined, lot specific value
Controls	1 each, positive and negative control intended to monitor for substantial reagent failure.	1 each, positive and negative control intended to monitor for substantial reagent failure.

Method of calibration	Intra-Well Calibration Technology	Calibration performed with calibrators in separate wells.
Intended Use	Qualitative assay using the other EBV markers for categorization of disease.	Qualitative assay using the other EBV markers for categorization of disease.
Automated Instrumentation	AtheNA Multi-Lyte® instrument - (Luminex® LS-100)	MAGO Plus® Automated EIA processor
Differences		
Item	Device	Predicate
Methodology	The AtheNA multiplex EBV test system is designed to detect three IgG class antibodies in human sera in one kit.	Diamedix EBV VCA IgG, EBNA IgG, EA IgG ELISA are provided in three separate kits for differentiation of each analyte.
Conjugate	Phycoerythrin-conjugated goat anti-human IgG (Fc chain specific)	Immunoperoxidase-conjugated Goat anti-human IgG
Substrate	The suspension contains separate distinguishable polystyrene beads conjugated with following antigens: affinity purified EBV VCA gp125, affinity purified EA (roughly equal parts EA-D and EA-R) and recombinant EBNA-1. 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	The respective EBV virus is coated on separate wells in a microwell plate(s).

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

L. Test Principle: The Zeus Scientific, Inc. AtheNA Multi-Lyte EBV test system is designed to detect IgG class antibodies in human sera to a variety of EBV antigens. The test procedure involves two incubation steps:

Test sera (properly diluted) are incubated in a vessel containing a multiplexed mixture of the bead suspension. The multiplexed bead suspension contains a mixture of distinguishable sets of polystyrene microspheres; three of these bead sets are conjugated with the three EBV antigens (EBV VCA, EBNA-1 and EBV EA). The bead mix also contains one bead set

designed to detect non-specific binding and four separate bead sets used for assay calibration. If present in patient sera, specific antibodies will bind to the immobilized antigen on one or more of the bead sets. The microspheres are rinsed to remove non-reactive serum proteins.

Phycoerythrin-conjugated goat anti-human IgG (Fc chain 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 convert raw fluorescence into outcome (units).

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. Precision/Reproducibility:

      Precision was evaluated at all three clinical sites. To evaluate both intra-assay and inter-assay reproducibility, six specimens were tested. On each day of testing, each sample was diluted twice and then loaded for four replicates resulting in a total of eight wells of each of the six samples. This protocol was followed for three days. These results were then used to calculate mean U/mL values, standard deviations, and percent CV. At each site, specimens were selected in such a way that resulted in some of them being clearly negative, some being clearly positive and some were selected that were weakly positive or just near the cut off of the assay. A summary of this testing appears above. In addition, precision testing was performed with reference serum.

Summary of Precision Testing:

EBV VCA

Panel Member	Mean U/mL	Site	Within Run Day 1		Within Run Day 2		Within Run Day 3		Between Day		Between Sites	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
1	603	1	20.12	3.4%	28.17	4.7%	50.64	8.3%	35.01	5.8%	42.18	7.2%
2	481	1	16.88	3.5%	27.84	5.9%	44.12	9.1%	30.73	6.4%	41.99	8.7%
3	80	1	13.15	14.6%	4.77	5.9%	5.96	8.5%	11.76	14.7%	12.37	14.8%
4	96	1	11.09	10.7%	7.61	8.3%	7.44	8.1%	10.47	10.9%	27.38	25.1%
5	43	1	3.62	8.0%	2.83	6.8%	3.29	7.8%	3.55	8.2%	6.21	17.2%
6	44	1	4.62	10.4%	4.14	9.3%	5.68	13.0%	4.66	10.5%	6.55	17.3%
1	559	2	22.23	4.1%	28.30	5.1%	28.79	5.0%	29.97	5.4%		
2	470	2	13.77	3.1%	12.24	2.7%	28.19	5.6%	33.14	7.1%		
3	77	2	5.78	8.4%	3.36	4.2%	3.62	4.3%	7.55	9.8%		
4	88	2	4.89	6.3%	7.02	7.8%	4.84	5.0%	9.62	10.9%		
5	32	2	2.56	8.1%	2.60	8.8%	2.39	6.9%	3.13	9.8%		
6	34	2	3.96	12.5%	2.07	6.6%	2.00	5.1%	4.49	13.2%		
1	584	3	27.63	4.3%	14.48	2.6%	22.82	4.1%	48.55	8.3%		
2	499	3	43.55	7.7%	20.07	4.3%	17.20	3.7%	54.35	10.9%		
3	94	3	5.59	5.3%	6.30	7.3%	7.69	8.5%	10.32	11.0%		
4	143	3	7.70	6.2%	9.57	6.4%	10.34	6.6%	15.98	11.2%		
5	33	3	2.67	7.1%	2.83	9.3%	2.73	8.8%	4.18	12.7%		
6	36	3	3.23	7.8%	4.16	12.3%	2.97	9.4%	5.32	15.0%		

EBNA

Panel Member	Mean U/mL	Site	Within Run Day 1		Within Run Day 2		Within Run Day 3		Between Day		Between Sites	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
7	941	1	55.26	6.0%	48.13	5.2%	53.18	5.4%	57.99	6.2%	45.14	4.9%
8	1041	1	39.14	3.8%	30.75	2.9%	76.73	7.5%	52.53	5.0%	55.95	5.4%
9	110	1	8.85	7.9%	18.41	16.1%	7.38	7.2%	12.94	11.8%	16.11	15.1%
10	43	1	5.42	11.4%	3.65	9.6%	5.15	12.1%	6.00	14.0%	8.00	20.6%
11	32	1	2.38	7.1%	3.00	9.3%	2.38	7.6%	2.62	8.1%	7.60	33.8%
12	23	1	2.93	11.9%	2.72	11.6%	2.45	11.4%	2.88	12.4%	6.88	47.7%
7	905	2	30.25	3.4%	12.96	1.4%	26.95	2.9%	31.76	3.5%		
8	1000	2	32.93	3.4%	24.48	2.5%	28.53	2.7%	59.45	5.9%		
9	99	2	6.58	7.6%	2.20	2.0%	4.57	4.4%	10.35	10.4%		
10	42	2	5.13	15.3%	4.32	10.0%	2.98	6.0%	7.84	18.7%		
11	18	2	1.39	7.2%	1.85	12.0%	1.49	7.5%	2.51	13.8%		
12	10	2	1.46	17.9%	2.07	21.8%	2.07	16.4%	2.64	26.1%		
7	931	3	20.77	2.2%	32.04	3.4%	27.81	3.1%	34.56	3.7%		
8	1046	3	45.47	4.2%	27.07	2.6%	43.14	4.2%	45.33	4.3%		
9	111	3	9.05	6.5%	8.10	8.4%	5.42	5.5%	21.08	19.0%		
10	32	3	5.55	18.5%	2.60	7.7%	4.17	13.3%	4.39	13.9%		
11	17	3	5.37	27.9%	3.16	20.5%	2.66	15.9%	4.08	23.8%		
12	10	3	3.14	24.4%	2.98	31.3%	2.49	32.2%	3.51	34.9%		

EA

Panel Member	Mean U/mL	Site	Within Run Day 1		Within Run Day 2		Within Run Day 3		Between Day		Between Sites	
			SD	% CV	SD	% CV	SD	% CV	SD	% CV	SD	% CV
13	785	1	48.68	6.3%	47.35	6.0%	86.79	10.9%	62.19	7.9%	70.85	9.9%
14	433	1	23.55	5.4%	37.22	8.9%	38.29	8.6%	34.38	7.9%	45.63	10.4%
15	100	1	6.65	6.5%	3.82	3.9%	11.97	12.0%	7.95	7.9%	13.70	12.6%
16	141	1	6.25	4.4%	14.79	10.1%	8.68	6.5%	11.40	8.1%	15.73	12.1%
17	78	1	5.62	6.8%	6.95	9.1%	11.20	14.9%	8.46	10.8%	23.07	47.9%
18	61	1	7.78	12.0%	8.94	14.6%	12.08	21.3%	9.94	16.3%	20.10	57.5%
13	687	2	20.68	3.0%	35.62	5.2%	42.69	6.2%	32.78	4.8%		
14	425	2	15.72	4.0%	24.87	6.2%	20.44	4.3%	40.73	9.6%		
15	119	2	4.72	4.3%	4.72	4.3%	7.45	5.5%	13.37	11.2%		
16	119	2	4.24	3.9%	5.70	4.7%	11.44	9.1%	10.22	8.6%		
17	35	2	4.75	14.1%	3.29	11.5%	6.25	14.6%	7.60	21.7%		
18	21	2	3.78	18.5%	3.78	18.0%	4.12	18.0%	3.88	18.1%		
13	677	3	26.82	3.6%	18.56	2.8%	39.96	6.4%	55.59	8.2%		
14	457	3	20.59	3.9%	19.33	4.6%	16.10	3.8%	54.87	12.0%		
15	107	3	6.32	6.6%	8.54	7.8%	11.08	9.6%	12.01	11.2%		
16	130	3	5.70	3.8%	4.49	3.8%	7.48	6.2%	16.54	12.7%		
17	31	3	8.67	20.5%	6.73	25.2%	5.96	23.7%	10.52	33.5%		
18	22	3	6.97	24.5%	9.13	40.8%	5.76	34.9%	8.68	38.6%		

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

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):* There is no traceability for the calibrators except for the cutoff having been set by testing a number of negative samples and setting the cutoff of +3 Standard Deviation (SD). The calibrators are set at a relatively low positive, mid positive and negative. These are utilized with Zeus' Intra-Well Calibration Technology; each well of the assay is calibrated internally without any user intervention. The standard curve is designed to self-adjust based upon the unique characteristics of the patient or control serum.

<li value="1">The Negative Control and the two Positive Controls must all be negative on the non-specific or control antigen bead
<li value="2">The Negative Control must be negative for each and every analyte included in the multiplexed bead suspension
<li value="3">The Positive Control must be positive for all of the three analytes included in the multiplexed bead suspension. These ranges are lot specific and are encoded within the Calibration CD. PC ranges may be viewed by clicking on the "Control Graphs" button of the AtheNA software and then clicking "Control Upper/Lower Limits"

    d. *Detection limit:* N/A

    e. *Analytical specificity:* Analytical specificity was based on comparison of this device to the established predicate, Diamedix.

    f. Assay cut-off: The cut off for each assay was established using a negative population for each marker. The AtheNA results were determined for this negative population, by establishing a cut off set at approximately the mean of the negative results plus three times the standard deviation (SD).

2. <u>Comparison studies:</u>

    a. *Method comparison with predicate device:*

For purposes of percent agreement calculations, the Athena EBV IgG equivocal results were assigned to the opposite clinical interpretation than that of the comparative assay result. Likewise, the comparative assay equivocal results were assigned to the opposite clinical interpretation than that of the AtheNA EBV IgG result. The percent agreement between the AtheNA EBV IgG assays and the comparative EBV IgG ELISA assays are summarized in the following tables by specimen EBV classification:

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV VCA IgG Assay vs. EBV VCA IgG Reference ELISA Assay

Prospective Specimens:

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	9.1%(1/11)	0% – 26.1%	94.1%(16/17)	82.9% – 100%
No infection	97.9%(92/94)	95.0% - 100%	N/A[c]	N/A
Past infection	N/A	N/A	91.9%(441/480)	89.4% - 94.3%
Indeterminate	44.4%(12/27)	25.7% - 63.2%	90.5%(57/63)	83.2% - 97.7%
Overall	78.9%(105/133)	72.0% - 85.9%	91.8%(514/560)	89.5% - 94.1%

a   x = the number of AtheNA EBV VCA IgG results that are confirmed positive in agreement with the reference EBV VCA IgG confirmed positive results; n = the total number of reference EBV VCA IgG results that are confirmed positive

b   x = the number of AtheNA EBV VCA IgG results that are nonreactive in agreement with the reference EBV VCA IgG; n = the total number of reference EBV VCA IgG results that are nonreactive

c   Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBNA IgG Assay vs. EBNA IgG Reference ELISA Assay

Prospective Specimens:

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	85.2%(23/27)	71.8% – 98.6%	N/A[c]	N/A
No infection	95.7%(90/94)	91.7% - 99.8%	N/A	N/A
Past infection	N/A	N/A	98.3%(472/480)	97.2% - 99.5%
Indeterminate	53.3%(24/45)	38.8% - 67.9%	91.1%(41/45)	82.8% - 99.4%
Overall	77.0%(137/178)	70.8% - 83.2%	97.7%(513/525)	96.4% - 99.0%

a   x = the number of AtheNA EBNA IgG results that are confirmed positive in agreement with the reference EBNA IgG confirmed positive results; n = the total number of reference EBNA IgG results that are confirmed positive

b   x = the number of AtheNA EBNA IgG results that are nonreactive in agreement with the reference EBNA IgG; n = the total number of reference EBNA IgG results that are nonreactive

c   Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV EA IgG Assay vs. EBV EA IgG Reference ELISA Assay

Prospective Specimens:

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	79.2%(19/24)	62.9% – 95.4%	25.0%(1/4)	0% – 67.4%
No infection	97.9%(93/95)	95.0% - 100%	N/A[c]	N/A
Past infection	55.8%(213/382)	50.8% - 60.7%	96.9%(95/98)	93.5% - 100%
Indeterminate	78.7%(59/75)	69.4% - 87.9%	86.7%(13/15)	69.5% - 100%
Overall	66.7%(384/576)	62.8% - 70.5%	93.2%(109/117)	88.6% - 97.7%

a x = the number of AtheNA EBV EA IgG results that are confirmed positive in agreement with the reference EBV EA IgG confirmed positive results; n = the total number of reference EBV EA IgG results that are confirmed positive

b x = the number of AtheNA EBV EA IgG results that are nonreactive in agreement with the reference EBV EA IgG; n = the total number of reference EBV EA IgG results that are nonreactive

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV VCA IgG Assay vs. EBV VCA IgG Reference ELISA Assay

Retrospective Specimens - Expected Acute

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	6.3%(1/16)	0% – 18.1%	100%(34/34)	100% – 100%
No infection	0.0%(0/1)	0% - 0%	N/A[c]	N/A
Past infection	N/A	N/A	100%(3/3)	100% - 100%
Indeterminate	11.1%(1/9)	0% - 31.6%	85.7%(6/7)	59.8% - 100%
Overall	7.7%(2/26)	0% - 17.9%	97.7%(43/44)	93.3% - 100%

a x = the number of AtheNA EBV VCA IgG results that are confirmed positive in agreement with the reference EBV VCA IgG confirmed positive results; n = the total number of reference EBV VCA IgG results that are confirmed positive

b x = the number of AtheNA EBV VCA IgG results that are nonreactive in agreement with the reference EBV VCA IgG; n = the total number of reference EBV VCA IgG results that are nonreactive

c Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBNA IgG Assay vs. EBNA IgG Reference ELISA Assay

Retrospective Specimens - Expected Acute

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	96.0%(48/50)	90.6% – 100%	N/A[c]	N/A
No infection	100%(1/1)	100% - 100%	N/A	N/A
Past infection	N/A	N/A	100%(3/3)	100% - 100%
Indeterminate	100%(4/4)	100% - 100%	8.3%(1/12)	0% - 24.0%
Overall	96.4%(53/55)	91.4% - 100%	26.7%(4/15)	4.3% - 49.0%

a   x = the number of AtheNA EBNA IgG results that are confirmed positive in agreement with the reference EBNA IgG confirmed positive results; n = the total number of reference EBNA IgG results that are confirmed positive

b   x = the number of AtheNA EBNA IgG results that are nonreactive in agreement with the reference EBNA IgG; n = the total number of reference EBNA IgG results that are nonreactive

c   Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

Percent Agreement and Confidence Intervals by EBV Classification; AtheNA EBV EA IgG Assay vs. EBV EA IgG Reference ELISA Assay

Retrospective Specimens - Expected Acute

EBV Classification	Negative Percent Agreement % (x/n)[b]	95% Exact Confidence Interval	Positive Percent Agreement % (x/n)[a]	95% Exact Confidence Interval
Acute	80.6%(25/31)	66.7% – 94.6%	21.1%(4/19)	2.7% – 39.4%
No infection	100%(1/1)	100% - 100%	N/A[c]	N/A
Past infection	50.0%(1/2)	0% - 100%	100%(1/1)	100% - 100%
Indeterminate	100%(10/10)	100% - 100%	16.7%(1/6)	0% - 46.5%
Overall	84.1%(37/44)	73.3% - 94.9%	23.1%(6/26)	6.9% - 39.3%

a   x = the number of AtheNA EBV EA IgG results that are confirmed positive in agreement with the reference EBV EA IgG confirmed positive results; n = the total number of reference EBV EA IgG results that are confirmed positive

b   x = the number of AtheNA EBV VCA IgM results that are nonreactive in agreement with the reference EBV EA IgG; n = the total number of reference EBV EA IgG results that are nonreactive

c   Agreement resulted in 0/0 specimens. In such cases, percent agreement and 95% confidence intervals could not be calculated.

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):

   A multisite comparative study was performed to evaluate the performance of the Zeus Scientific, Inc., AtheNA Multi-Lyte EBV IgG test system to the disease classification of the specimens as determined by other EBV serological reagents. Specimens were tested by ELISA for EBV VCA IgG, EBNA IgG, EBV VCA IgM and for heterophile antibody using a latex agglutination assay for purposes of classification into disease states. EBV EA IgG ELISA results were not considered for purposes of classification of specimens into disease states.   There were a total of 763 specimens tested. Of the 763 specimens tested, 693 were prospective specimens and 70 were retrospective specimens. The retrospective group was selected since clinical information suggested that they were representative of acute cases of infectious mononucleosis.

   Tables were created based on a hypothetical samples tested according to previous clinical data in the form of EBV disease category incorporated into a table.

EBV Classification	Heterophile	VCA IgM	VCA IgG	EBNA-1 IgG	EBV EA IgG[1]
Acute infection	+	+	+	−	+
	+	+	−	−	+
	−	+	+	−	+
	+	+	+	−	n/a
	+	+	−	−	n/a
	−	+	+	−	n/a
No infection	−	−	−	−	−
	n/a	−	−	−	−
Past infection	−	−	+	+	+
	n/a	−	+	+	+
	−	−	+	+	n/a
	n/a	−	+	+	n/a
Indeterminate	Any combination not noted in the three categories above.				

[1] The EA antigen used for AtheNA contains roughly equal parts of EA/D and EA/R.   Anti-EA/D shows a transient rise during acute infection, undetectable after 3 – 6 months.   Anti-EA/R appears after EA/D and may be present greater than or equal to 2 years.

+ = Reactive
− = Nonreactive
n/a = not available

4. Clinical cut-off: Because there was no clinical cutoff established per se, but only use of the above table to determine disease categorization, the cutoff was determined by testing negative samples and establishing an equivocal zone around the cutoff based on 3 standard deviations thereof. The testing is controlled by three calibrators, two controls and evaluating a sample by testing multiple analytes.

5. Expected values/Reference range: Because there was no clinical information available on the samples tested, the most that could be established is the prevalence of the sex of each marker tested compared to comparable literature studies.

EBV VCA IgG:

For the female group, 81.1% (167/206) were positive, 18.0% (37/206) were negative, 1.0% (2/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 78.7% (107/136) were positive, 19.9% (27/136) were negative, 1.5% (2/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of 763 specimens tested, 606/763 (79.4%) were positive, 148/763 (19.4%) were negative, 8/763 (1.0%) were equivocal and 1/763 (0.1%) were invalid.

EBNA IgG:

For the female group, 86.4% (178/206) were positive, 12.1% (25/206) were negative, 1.5% (3/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 86.8% (118/136) were positive, 13.2% (18/136) were negative, 0% (0/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of

763 specimens tested, 547/763 (71.7%) were positive, 211/763 (27.7%) were negative, 4/763 (0.5%) were equivocal and 1/763 (0.1%) were invalid.

EBV EA IgG:

For the female group, 49.5% (102/206) were positive, 46.6% (96/206) were negative, 3.9% (8/206) were equivocal and 0% (0/206) yielded invalid results. For the male group, 49.3% (67/136) were positive, 45.6% (62/136) were negative, 5.1% (7/136) were equivocal and 0% (0/136) yielded invalid results. With respect to the entire population of 763 specimens tested, 283/763 (37.1%) were positive, 452/763 (59.2%) were negative, 27/763 (3.5%) were equivocal and 1/763 (0.1%) were invalid.

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.