

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K093784

**B. Purpose for Submission:**

New device

**C. Measurand:**

IgG class antibodies to *Toxoplasma gondii* (*T. gondii*), Rubella, Cytomegalovirus (CMV) and Herpes Simplex virus (HSV 1 and HSV 2).

**D. Type of Test:**

Multiplex flow immunoassay (multiplexed fluoromagnetic bead assay)

**E. Applicant:**

Zeus Scientific Inc.

**F. Proprietary and Established Names:**

Proprietary Name: AtheNA Multi-Lyte<sup>®</sup> ToRCH IgG Plus Test System

Established name: Toxoplasma, Rubella, Cytomegalovirus, Herpes Simplex 1 and Herpes Simplex 2 serological reagent

**G. Regulatory Information:**

<b>Product code</b>	<b>Classification</b>	<b>Regulation section</b>	<b>Panel</b>
OPM: Multiplex immunoassay for <i>T. gondii</i> , Rubella, Cytomegalovirus and Herpes Simplex virus 1 and 2	Class II	866.3510; Rubella Virus Serological Reagents	Microbiology

Note: The AtheNA Multi-Lyte<sup>®</sup> ToRCH IgG Plus Test System is a multiplex immunoassay for the detection of IgG antibodies to *T. gondii*, Rubella, CMV, HSV 1 and HSV 2. This device is classified as Class II as described above and the new product code assigned for this device is listed under the regulation section for Rubella reagents. The classification of the panel follows that of Rubella serological reagents. The following is a list of the other regulation sections and product codes that are applicable to the individual analytes detected by the device subject of this submission.

1. 866.3780; Toxoplasma gondii serological reagents (Microbiology Panel: Class II). Product code (LGD), Enzyme linked immunoabsorbent assay, Toxoplasma gondii
2. 866.3175; Cytomegalovirus serological reagents (Microbiology Panel: Class II). Product code (LFZ), Enzyme linked immunoabsorbent assay, Cytomegalovirus
3. 866.3305; Herpes Simplex 1 serological reagents (Microbiology Panel: Class II). Product code (MXJ), Enzyme linked immunoabsorbent assay, Herpes Simplex Virus, HSV 1
4. 866.3305; Herpes Simplex 2 serological reagents (Microbiology Panel: Class II). Product code (MYF), Enzyme linked immunoabsorbent assay, Herpes Simplex Virus, HSV 2

**H. Intended Use:**

1. Intended use(s):

**AtheNA Multi-Lyte® ToRCH IgG Plus Test System**

The Zeus Scientific, Inc. AtheNA Multi-Lyte® ToRCH IgG Plus Test System is intended for the qualitative detection of specific human IgG class antibodies to Toxoplasma gondii (*T. gondii*), Rubella, Cytomegalovirus (CMV) and HSV 1 & 2 in human serum. The results of this assay are intended to be used as an aid in the assessment of serological status to Toxoplasma gondii, Rubella and CMV. For HSV 1 and HSV 2, 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 test is not intended for use in screening blood or plasma donors.

The performance of this assay has not been established for use in a pediatric population, neonatal screening, immunocompromised or immunosuppressed patients or for use at point of care facilities.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

The AtheNA Multi-Lyte® System

## I. Device Description:

The Zeus Scientific, Inc. AtheNA Multi-Lyte<sup>®</sup> ToRCH IgG Plus Test System is a multiplex immunoassay intended for the simultaneous qualitative detection and differentiation of specific human IgG class antibodies to *T. gondii*, Rubella, CMV, HSV 1 and HSV 2 in human serum using the Luminex flow cytometry technology. The test consists of the AtheNA Multi-Lyte reagent kit, software and the Luminex Corp instrument. The reagent kit consists of the following reagents in sodium azide preservative;

1. Multiplexed bead suspension containing separate distinguishable 5.6 micron polystyrene beads that are conjugated with; Toxoplasma grade 2 antigen, Rubella K2S grade antigen, CMV grade 2, HSV-1 type-specific recombinant gG-1 protein antigen and HSV-2 gG-2 type-specific recombinant gG-2 protein antigen. 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.
2. Conjugate: Phycoerythrin conjugated goat anti-human IgG ( $\gamma$  chain specific).
3. Human positive serum control
4. Human positive serum control
5. Human negative serum control.
6. SAve Diluent<sup>®</sup> containing phosphate-buffered-saline.
7. Wash Buffer Concentrate
8. One, 96-well filtration plate for rinsing the microspheres
9. Data Labels
10. Package Insert providing instructions for use
11. Calibration CD: a compact disc that includes all lot-specific kit calibration values required for specimen analysis and assay quality control.

## J. Substantial Equivalence Information:

1. Predicate device name(s):

The following is a list of the predicate devices and reference methods (as applicable) for each of the analytes in the panel. These predicate devices are used as comparators to assess the performance of the device for each of the analytes in the panel. The final classification of the new multiplexed device follows that of the Rubella predicate device.

Zeus Scientific, Inc: Toxo IgG ELISA Test System  
Zeus Scientific, Inc: Rubella IgG ELISA Test System  
Zeus Scientific, Inc: CMV IgG ELISA Test System  
Focus Diagnostics: HerpeSelect 1 and 2 Immunoblot IgG (reference method for this indication)

2. Predicate 510(k) number(s):

The 510(k) numbers for the predicate devices for *T. gondii*, Rubella, CMV and HSV 1 & 2 are K891781, K891783, K924096 and K000238.

3. Comparison with predicates:

1. Comparison with Zeus Scientific, Inc: Rubella IgG ELISA Test System

<b>Similarities</b>		
Item	Device	Predicates
Intended use/ Indications for Use	The Zeus Scientific, Inc. AtheNA Multi-Lyte <sup>®</sup> ToRCH IgG Plus Test System is intended for the <b>qualitative detection of specific human IgG class antibodies to Toxoplasma gondii (T. gondii), Rubella, Cytomegalovirus (CMV) and HSV 1 &amp; 2</b> in human serum. The results of this assay are intended to be used <b>as an aid in the assessment of serological status</b> to Toxoplasma gondii, Rubella and CMV. For HSV 1 and HSV 2, 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 Zeus Scientific, Inc: Rubella IgG ELISA Test System is intended for the <b>qualitative</b> and/or quantitative detection of <b>IgG antibodies to Rubella</b> in human sera. Intended to be used to <b>evaluate single sera for immune status</b> or paired sera to demonstrate seroconversion.
Measurand	<i>T. gondii</i> , <b>Rubella</b> , CMV in addition to HSV 1 and HSV 2 IgG antibodies	Rubella IgG
Detection	<b>Qualitative</b> detection	<b>Qualitative</b> and quantitative Detection
Matrix	Serum	same

<b>Differences</b>		
Item	Device	Predicate
Analytes detected	Multiple Analytes	Single Analyte
Calibrators	Multiple calibrators	Standard
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme Linked immunosorbent assay (ELISA), using Antigen coated plates

2. Comparison with Zeus Scientific, Inc: Toxo IgG ELISA Test System

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
Intended Use/ indications	Intended for the <b>qualitative detection of specific human IgG class antibodies to Toxoplasma gondii (<i>T.gondii</i>)</b> , Rubella, Cytomegalovirus (CMV) and HSV 1 & 2 in human serum. The results of this assay are intended to be used <b>as an aid in the assessment of serological status</b> to Toxoplasma gondii, Rubella and CMV.	Intended for the <b>qualitative</b> and/or quantitative detection of <b>IgG antibodies to <i>T.gondii</i></b> in human sera. Intended to be used to <b>evaluate serologic evidence of past infection with <i>T. gondii</i></b>
Measurand	<b><i>T.gondii</i></b> , Rubella, CMV in addition to HSV 1 and HSV 2 IgG antibodies	<i>T.gondii</i> IgG
Detection	Qualitative detection	<b>Qualitative</b> and quantitative Detection
Matrices	Serum	Serum

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Analytes detected	Multiple Analytes	Single Analyte
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme Linked immunosorbent assay (ELISA), using Antigen coated plates

3. Comparison with Zeus Scientific, Inc: CMV IgG ELISA Test System

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
Intended Use/ Indications for use	Intended for the <b>qualitative detection of specific human IgG class antibodies to Toxoplasma gondii (<i>T.gondii</i>)</b> , Rubella, <b>Cytomegalovirus (CMV)</b> and HSV 1 & 2 in human serum. The	Intended for the <b>qualitative</b> detection of <b>IgG antibodies to CMV</b> in human sera. Intended to be used to <b>evaluate serologic evidence of past infection with CMV</b>

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicates</b>
	results of this assay are intended to be used as <b>an aid in the assessment of serological status</b> to Toxoplasma gondii, Rubella and CMV.	
Measurand	<b>T.gondii</b> , Rubella, CMV in addition to HSV 1 and HSV 2 IgG antibodies	CMV IgG
Detection	Qualitative detection	<b>same</b>
Matrix	Serum	<b>same</b>

<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Analytes detected	Multiple Analytes	Single Analyte
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Enzyme Linked immunosorbent assay (ELISA), using Antigen coated plates

4. Focus Diagnostics: HerpeSelect 1 and 2 Immunoblot IgG (reference method for this indication)

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Indications for use	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	same

Similarities		
Item	Device	Predicate
	facilities or for use with automated equipment.	
matrix	serum	same
measurand	T.gondii, Rubella, CMV in addition to <b>HSV 1 and HSV 2 IgG antibodies</b>	HSV 1 and HSV 2 IgG antibodies
antigen	<ol style="list-style-type: none"> <li>1. Recombinant gG1 antigen (molecular weight 55 KD)</li> <li>2. Recombinant gG2 antigen (molecular weight 31 KD)</li> </ol>	<ol style="list-style-type: none"> <li>1. HSV native virus antigens</li> <li>2. Recombinant gG1 antigen 35-45 KD</li> <li>3. Recombinant gG2 antigen 80-110 KD</li> </ol>

Differences		
Item	Device	Predicate
Analytes detected	Multiple Analytes	Single Analyte
Technology	Multiplexed flow immunoassay, using Antigen-coated paramagnetic microbead reagent.	Immunoblot Assay

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

CLSI guideline; Procedures for the Handling and Processing of Blood Specimens; Approved Guideline-Second Edition (H-18 A-3)

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

**L. Test Principle:**

The Zeus Scientific, Inc. AtheNA Multi-Lyte ToRCH IgG Plus test procedure involves two incubation steps:

1. Patient sera are diluted and the diluted test sera 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. Conjugated to the primary sets of microspheres are *Toxoplasma*, Rubella, CMV and HSV 1 & 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, the individual 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 beads in step 1. The bead suspension is then analyzed by the AtheNA Multi-Lyte instrument. The bead set(s) are sorted and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using the Intra-Well Calibration Technology<sup>®</sup>, internal calibration bead sets are used to establish the assay's cutoff. Raw fluorescence from the distinct antigen bead is measured and compared against the cut-off calibrator

#### **M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

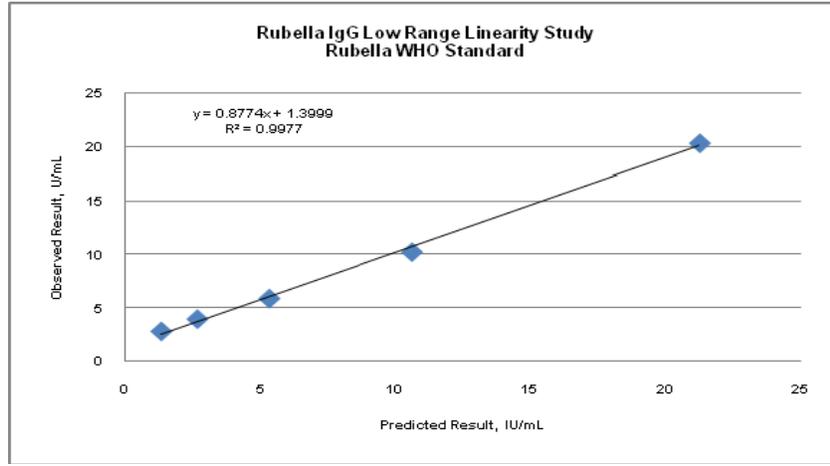
*a. Precision/Reproducibility:*

Assay precision and reproducibility was evaluated internally and at two external clinical sites. The study was conducted as follows: Six samples were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study based upon their activity on the AtheNA Multi-Lyte assay. Two samples was selected that were clearly negative, two that were clearly positive and two samples that were near the assay cut off. To assess reproducibility, on each day of testing, each sample was diluted twice and each dilution was run in triplicate. This resulted in six results per day. This was repeated for three days at each site and the resulting data used to assess precision at each facility. The studies are summarized in the table below:

Panel Member	Sample N	Mean AU/mL	Within-Run		Between-Day		Between-Run		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Toxo IgG Positive 1	54	747.2	52.1	7.3	61.6	8.5	34.5	4.7	71.5	8.7	157.2	8.0
Toxo IgG Positive 2	54	770.3	50.8	6.8	63.1	8.3	39.2	4.9	78.7	8.5	170.3	8.6
Toxo IgG Positive 1 (near Cut-off)	54	158.8	158.8	9.3	18.2	11.3	12.6	8.0	18.8	12.1	29.5	11.9
Toxo IgG Positive 2 (near Cut-off)	54	140.2	140.2	8.4	16.8	11.3	13.0	8.3	20.2	12.1	33.2	12.9
Toxo IgG Negative 1	54	10.5	10.5	40.9	3.8	39.7	1.3	15.0	4.3	38.4	5.0	40.7
Toxo IgG Negative 2	54	9.1	9.1	45.4	4.1	47.4	2.4	22.6	4.7	48.0	5.6	49.8
Rubella IgG Positive 1	54	1296.9	50.1	3.9	68.4	5.0	54.5	3.7	95.4	5.3	301.3	5.7
Rubella IgG Positivitive 2	54	1102.6	58.2	5.4	63.6	5.8	37.3	3.4	99.5	6.5	173.1	7.0
Rubella IgG Positive 1 (near Cut-off)	54	242.8	13.3	5.4	15.6	6.5	10.2	4.4	25.6	6.2	51.9	6.4
Rubella IgG Positive 2 (near Cut-off)	54	189.9	10.1	5.2	15.5	9.5	11.5	5.7	28.3	10.1	37.9	10.4
Rubella IgG Negative 1	54	27.9	4.8	20.5	16.1	21.9	1.8	8.3	4.2	21.3	19.4	19.3
Rubella IgG Negative 2	54	47.4	4.0	10.3	6.5	9.8	3.7	9.4	8.8	10.9	23.8	11.0
CMV IgG Positive 1	54	996.5	88.2	8.5	99.2	9.7	58.5	5.9	108.2	10.1	167.2	10.0
CMV IgG Positive 2	54	756.9	54.5	7.0	66.7	8.7	46.8	6.3	73.3	8.8	1.6.3	8.7
CMV IgG Positive 1 (near Cut-off)	54	119.2	10.1	8.2	13.2	11.1	9.6	8.3	14.9	10.9	18.3	10.1
CMV IgG Positive 2 (near Cut-off)	54	135.3	13.6	9.9	16.4	12.1	12.6	9.4	19.5	10.7	21.2	10.8
CMV IgG Negative 1	54	17.9	5.6	29.8	6.1	32.7	3.4	19.5	6.3	28.2	7.4	27.1
CMV IgG Negative 2	54	16.4	5.5	35.5	5.9	37.1	3.6	23.4	6.3	35.4	7.3	35.4
HSV 1 IgG Positive 1	54	310.1	24.2	7.9	24.8	8.1	10	3.3	31	8.9	32.3	10.1
HSV 1 IgG Positive 2	54	392.7	31.5	8.0	32.3	8.2	15.6	3.8	48.1	8.7	50.8	8.4
HSV 1 IgG Positive 1 (near Cut-off)	54	144.6	15.4	10.7	17.2	12.0	8.9	6.0	22.3	12.4	23.0	12.4
HSV 1 IgG Positive 2 (near Cut-off)	54	191.7	17.6	9.1	19.7	10.2	9.6	5.1	24.5	9.9	27.4	10.6
HSV 1 IgG Negative 1	54	26.4	3.9	15.5	3.9	15.5	1.4	5.2	4.8	16.2	6.1	16.8
HSV 1 IgG Negative 2	54	8.2	2.3	30.7	2.5	34.1	1.3	17.0	2.7	36.9	3.5	39.4
HSV 2 IgG Positive 1	54	445.7	26.9	6.1	40.1	8.9	33.8	7.4	53.4	9.2	58.1	9.1
HSV 2 IgG Positive 2	54	355.6	27.0	7.4	30.5	8.4	18.8	5.2	51.5	8	57.2	8.6
HSV 2 IgG Positive 1 (near Cut-off)	54	152.2	14.9	9.9	16.0	10.6	7.6	5.2	25.3	10.0	31.6	11.2
HSV 2 IgG Positive 2 (near Cut-off)	54	114.1	11.4	9.8	12.5	10.9	6.3	5.7	15.4	10.8	20.4	10.8
HSV 2 IgG Negative 1	54	16.9	4.0	29.7	4.3	31.3	2.0	12.1	4.3	35.8	7.0	41.0
HSV 1 IgG Negative 2	54	21.2	5.9	27.5	6.5	30.6	3.1	15.2	6.8	26.6	8.9	27.6

*b. Linearity/assay reportable range:*

The test is a qualitative assay and linearity data is not required. However, for Rubella the lower range of the assay and the range around the cut-off (0 – 20 IU/ml) is quantitative. The company demonstrated the linearity for this assay range using a titration of the World Health Organization (WHO) anti-Rubella immunoglobulin, 1st International Standard, 1996.



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

The test is a qualitative assay. However, for Rubella the lower range of the assay, the range around the cut-off (0 – 20 IU/ml) is traceable to the World Health Organization (WHO) anti-Rubella immunoglobulin, 1st International Standard, 1996. The percent recovery for the international standard is represented in the table below.

Expected Result IU/mL	AtheNA Multi-Lyte lot 1				AtheNA Multi-Lyte lot 2			
	Measured Mean		Mean % Recovery		Measured Mean		Mean % Recovery	
	Result	IU/mL	Result	IU/mL	Result	IU/mL	Result	IU/mL
1.33	3	2.7	210	205	3	2.7	231	205
2.66	4	3.6	148	137	4	3.6	146	137
5.31	6	5.5	110	103	6	5.5	105	103
10.63	10	9.1	96	86	11	10	102	94
21.25	20	18.2	96	86	20	18.2	92	86

d. *Detection limit:*

Not applicable as this assay is a qualitative assay.

e. *Analytical specificity:*

Cross-Reactivity

Studies were performed at the manufacturing facility to assess cross reactivity with the Athena Multi-Lyte ToRCH IgG Plus test system using samples that

were sero-positive to Measles, Mumps, Rubella, VZV, EBV VCA IgG, EBNA-1, HSV-1, HSV-2, CMV, Syphilis, Toxoplasma and ANA and Rf IgM. Micro-particle and ELISA immunoassay test systems manufactured for commercial distribution were used to determine the sero-positivity of the samples. Ten samples minimally for each possible cross-reactant were tested. The results presented were obtained by testing the analytes against high concentrations of possible cross reactants.

<b>AtheNA Multi-Lyte ToRCH IgG Plus Cross Reactivity Summary (Samples Positive/Samples Tested)</b>					
<b>Analyte</b>	<b>Toxoplasma</b>	<b>Rubella</b>	<b>CMV</b>	<b>HSV 1</b>	<b>HSV 2</b>
<b>Measles</b>	0/10	0/20	0/10	0/10	0/10
<b>Mumps</b>	0/10	0/20	0/10	0/10	0/10
<b>Rubella</b>	0/10	N/A	0/10	0/10	0/10
<b>VZV</b>	0/10	0/20	0/10	0/10	0/10
<b>VCA IgG</b>	0/10	0/20	0/10	0/10	0/10
<b>EBNA IgG</b>	0/10	0/20	0/10	0/10	0/10
<b>HSV 1</b>	0/10	0/20	0/10	NA	0/10
<b>HSV 2</b>	0/10	0/20	0/10	0/10	NA
<b>ANA</b>	0/10	0/4	0/10	0/10	0/10
<b>RF</b>	0/10	0/10	0/10	0/10	0/10
<b>CMV</b>	0/10	0/20	NA	0/10	0/10
<b>Syphilis</b>	0/10	0/10	0/10	0/10	0/10
<b>Toxoplasma</b>	NA	0/20	0/10	0/10	0/10

### Interfering Substances

The effect of potential interfering substances on sample results generated using the AtheNA Multi-Lyte test system was evaluated with the following possible interfering substances based on the guidelines established in CLSI EP7-A2 (39): albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids.

The quantity of analyte in each 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: 20 g/dL (low), 20 g/dL (high)

Intralipid: 300 mg/dL (low), 750 mg/dL (high)

Three samples each for Toxo, Rubella, CMV, HSV 1 and 2 IgG were chosen based on their performance on the AtheNA Multi-Lyte test system: positive, borderline and negative. The samples were diluted to concentrations around the cut off, and tested with the possible interfering substances at the specified high and low concentrations. All samples showed less than a 20% change in signal with the exceptions presented in table 13. The use of samples which contain elevated levels of bilirubin, albumin, cholesterol, triglycerides, hemoglobin or intralipid should thus be avoided. The use of such samples may interfere with the outcome of the sample's result.

Analyte/Level of Sample	Potential Interfering Substance Spikes Exhibiting Change in Signal Greater than 20%											
	Bilirubin		Albumin		Cholesterol		Triglycerides		Hemoglobin		Intralipid	
	High	Low	High	Low	High	Low	High	Low	High	Low	High	Low
Toxoplasma Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	22%	<20%	<20%
Toxoplasma Borderline	<20%	<20%	-33%	-30%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
Toxoplasma Negative	<20%	<20%	78%	89%	31%	<20%	<20%	<20%	<20%	22%	78%	<20%
Rubella Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	24%	<20%	<20%
Rubella Borderline	<20%	<20%	<20%	<20%	-27%	<20%	<20%	<20%	<20%	-31%	<20%	<20%
Rubella Negative	50%	50%	<20%	-33%	<20%	-33%	-33%	-33%	<20%	-33%	<20%	<20%
CMV Positive	<20%	<20%	-27%	-25%	-32%	-31%	<20%	<20%	-33%	-35%	<20%	<20%
CMV Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	31%	24%	<20%
CMV Negative	<20%	25%	<20%	32%	<20%	<20%	<20%	<20%	<20%	25%	<20%	25%
HSV 1 Positive	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	27%	22%	<20%	<20%
HSV 1 Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
HSV 1 Negative	<20%	<20%	41%	36%	<20%	<20%	<20%	20%	56%	26%	<20%	<20%
HSV 2 Positive	<20%	<20%	<20%	<20%	27%	<20%	24%	21%	<20%	23%	<20%	<20%
HSV 2 Borderline	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%	<20%
HSV 2 Negative	50%	41%	<20%	<20%	<20%	<20%	<20%	<20%	32%	35%	<20%	<20%

*f. Assay cut-off:*

A minimum of 25 samples were tested using the predicate ELISA test systems and confirmed as negative and then tested with the investigational device. Using the mean and standard deviations of this negative population for T. gondii, Rubella, CMV, HSV1 and HSV2, theoretical cut-off was calculated. Known positive samples, selected for their reactivity with the predicate devices, were tested with the investigational device and checked to ascertain that the results fell above the theoretical cut-off.

The cut-off values were verified using data obtained from clinical specimens 381 negative *T. gondii* samples, 22 negative Rubella samples, 212 negative CMV samples, 176 negative HSV1 samples and 304 negative HSV2 samples (the reactivity of the samples based upon results obtained from an alternate FDA cleared test system approved for commercial distribution), the mean was calculated and multiplied by the correction factor obtained in establishment of the cut-off. The values obtained approximate the assay cut-off, thus verifying that the method used to establish the cut-off for the AtheNA ToRCH IgG Plus Test System is valid.

2. Comparison studies:

a. *Method comparison with predicate device:*

See clinical studies

b. *Matrix comparison:*

Not Applicable

Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical specificity:*

Not Applicable

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

Comparative Testing: Prospective

Performance of the Athena Multi- Lyte ToRCH IgG system was evaluated against commercially available assays for Toxoplasma, Rubella, CMV, HSV1 and HSV 2 assays using prospectively collected frozen remnant serum samples from a total of 651 individuals for which ToRCH IgG panel or testing for each of the individual analytes was ordered. Two outside investigators tested 300 and 351 samples respectively. Results of this comparative study are summarized in the table below.

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
AtheNA Multi-Lyte IgG Plus	<b>Toxoplasma</b>						
	Positive	136	3	16	155	99.3% (136/137)	96.0% - 100%
	Equivocal	0	0	3	3		
	Negative	0	1	490	491	95.7% (450/514)	98.0% - 99.9%
	Invalid	0	0	2	2		
	Site Total	136	4	511	651		
	<b>Rubella</b>						
	Positive	533	4	4	541	98.5% (533/541)	97.1% - 99.4%
	Equivocal	3	1	1	5		
	Negative	2	3	60	65	*87% (60/69)	76.7% - 93.9%
	Invalid	0	0	0	0		
	Site Total	538	8	65	611		
	<b>CMV</b>						
	Positive	450	6	6	462	99.6% (450/452)	98.4% - 100%
	Equivocal	1	2	4	7		
Negative	1	0	181	182	91.3% (181/197)	87.2% - 95.3%	
Invalid	0	0	0	0			
Site Total	452	8	191	651			

\*4/4 discrepant Rubella samples which tested positive by AtheNA and negative by ELISA had low positive values for AtheNA and high negative values for ELISA. 4/4 discrepant Rubella samples which tested positive by AtheNA and equivocal by ELISA had low positive values for AtheNA and high equivocal values for ELISA.

The performance of the HSV1 and HSV2 was evaluated in prospectively collected samples using results from 596/651 individuals between the ages of 17 and 69. Results of this comparative study are summarized in table below.

		Predicate					
		Positive	Equivocal	Negative	Site Total	Sensitivity Specificity	95% CI
AtheNA Multi-Lyte IgG Plus	<b>HSV 1</b>						
	Positive	418	0	8	426	98.6% (418/424)	97.0% - 99.5%
	Equivocal	4	0	1	5		
	Negative	2	0	163	165	94.6% (163/172)	90.3% - 97.6%
	Invalid	0	0	0	0		
	Site Total	424	0	172	596		
	<b>HSV 2</b>						
	Positive	127	0	27	154	96.9% (127/131)	92.4% - 98.8%
	Equivocal	1	0	3	4		
	Negative	3	0	433	436	93.5% (433/463)	90.9% - 95.6%
Invalid	0	0	0	0			
Site Total	131	0	463	594*			

Performance in Pregnant Women Population:

Zeus Scientific internally evaluated 200 frozen remnant serum samples collected from pregnant women between the ages of 15 and 46 for which ToRCH antibody testing was requested. Results of this comparative study are summarized in the table below.

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
AtheNA Multi-Lyte IgG Plus : Toxoplasma	<b>Toxoplasma</b>						
	Positive	22	1	6	29	100.0% (22/22)	87.3% - 100%
	Equivocal	0	0	1	1		
	Negative	0	0	170	170	95.3% (170/178)	91.3% - 98.0%
	Invalid	0	0	0	0		
	<b>Site Total</b>	<b>22</b>	<b>1</b>	<b>177</b>	<b>200</b>		
	<b>Rubella</b>						
	Positive	194	0	0	194	99.0% (194/196)	96.4% - 99.9%
	Indeterminate	1	0	0	1		
	Negative	0	1	4	5	100.0% (4/4)	47.3% - 100%
	Invalid	0	0	0	0		
	<b>Site Total</b>	<b>195</b>	<b>1</b>	<b>4</b>	<b>200</b>		
	<b>CMV</b>						
	Positive	151	0	0	151	98.1% (151/154)	94.4% - 99.6%
	Equivocal	0	0	0	0		
	Negative	0	3	46	49	100.0% (46/46)	93.7% - 100%
	Invalid	0	0	0	0		
	<b>Site Total</b>	<b>151</b>	<b>3</b>	<b>46</b>	<b>200</b>		
<b>HSV 1</b>							
Positive	137	0	8	145	99.3% (137/138)	96.1% - 100%	
Equivocal	0	0	0	0			
Negative	1	0	46	47	85.2% (46/54)	72.3% - 93.4%	
Invalid	0	0	0	0			
<b>Site Total</b>	<b>138</b>	<b>0</b>	<b>54</b>	<b>192</b>			
<b>HSV 2</b>							
Positive	68	0	7	75	97.1% (68/70)	90.1% - 99.7%	
Equivocal	0	0	2	2			
Negative	2	0	113	115	92.6% (113/122)	86.5% - 96.6%	
Invalid	0	0	0	0			
<b>Site Total</b>	<b>70</b>	<b>0</b>	<b>122</b>	<b>192</b>			

Comparative Testing: Retrospective

Rubella Retrospective Negative Sample Study:

The relative specificity of Rubella was assessed internally at Zeus using pre-selected banked samples of sera which previously tested negative for Rubella antibody by the predicate device. The results are presented in the following table.

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
<b>AtheNA Multi-Lyte IgG Plus</b>	<b>Rubella</b>						
	<b>Positive</b>	0	0	0	0	N/A	N/A
	<b>Indeterminate</b>	0	0	0	0		
	<b>Negative</b>	0	0	100	100	100.0% 100/100)	97.1% - 100%
	<b>Invalid</b>	0	0	0	0		
	<b>Site Total</b>	0	0	100	100		

HSV 1 & 2 Performance in a Low Prevalence Population:

The relative specificity of HSV 1 & 2 was assessed internally using sera from a low prevalence population. The low prevalence population was comprised of serum samples from 18 and 19 year old subjects previously tested for infections considered non-sexual in nature. The results are presented in the following table.

		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
<b>AtheNA Multi-Lyte IgG Plus</b>	<b>HSV 1</b>						
	<b>Positive</b>	8	0	2	10	100.0% (8/8)	68.8% - 100%
	<b>Equivocal</b>	0	0	0	0		
	<b>Negative</b>	0	0	56	56	96.6% (56/58)	88.1% - 99.6%
	<b>Invalid</b>	0	0	0	0		
	<b>Site Total</b>	8	0	58	66		
	<b>HSV 2</b>						
	<b>Positive</b>	3	0	1	4	100.0% (3/3)	36.8% - 100%
	<b>Equivocal</b>	0	0	0	0		
	<b>Negative</b>	0	0	62	62	98.4% (62/63)	91.5% - 100%
	<b>Invalid</b>	0	0	0	0		
	<b>Site Total</b>	3	0	63	66		

Correlation with CDC Rubella Evaluation Serum Panel:

The performance of the AtheNA Multi-Lyte ToRCH IgG Plus test system was assessed using masked, well characterized serum panel from the CDC. The panels consist of:

1. 70% Toxo positive and 30% Toxo negative samples.
2. 80% Rubella positive and 20% Rubella negative samples.
3. 54% CMV positive and 46% CMV negative samples.
4. 24% HSV1 and HSV2 dual-positive samples, 50% HSV1 positive and 50% HSV1 negative samples and 48% HSV2 positive and 52% HSV2 negative samples.

The results are presented to convey further information on the performance of the test kit and do not imply endorsement of the assay by the CDC. The results are presented below.

		CDC Result				
		Positive	Negative	Site Total	PPA NPA	95% CI
<b>AtheNA Multi-Lyte ToRCH IgG Plus</b>	<b>Toxoplasma</b>					
	<b>Positive</b>	70	0	70	100.0% (70/70)	95.8% - 100.0%
	<b>Equivocal</b>	0	0	0		
	<b>Negative</b>	0	30	30	100.0% (30/30)	90.5% - 100.0%
	<b>Invalid</b>	0	0	0		
	<b>Site Total</b>	70	30	100		
	<b>Rubella</b>					
	<b>Positive</b>	80	0	80	100.0% (80/80)	96.3% - 100.0%
	<b>Equivocal</b>	0	0	0		
	<b>Negative</b>	0	20	20	100.0% (20/20)	86.1% - 100.0%
	<b>Invalid</b>	0	0	0		
	<b>Site Total</b>	80	20	100		
	<b>CMV</b>					
	<b>Positive</b>	52	2	54	100.0% (52/52)	94.4% - 100.0%
	<b>Equivocal</b>	0	0	0		
<b>Negative</b>	0	46	46	95.8% (46/48)	90.2% - 100.0%	
<b>Invalid</b>	0	0	0			
<b>Site Total</b>	52	48	100			

HSV 1						
Positive	50	0	50	100.0% (50/50)	94.2% - 100.0%	
Equivocal	0	0	0			
Negative	0	50	50	100.0% (50/50)	94.2% - 100.0%	
Invalid	0	0	0			
Site Total	50	50	100			
HSV 2						
Positive	48	1	49	100.0% (48/48)	94.0% - 100.0%	
Equivocal	0	0	0			
Negative	0	51	51	98.1% (51/52)	94.3% - 100.0%	
Invalid	0	0	0			
Site Total	48	52	100			

CDC Rubella Low Positive Control Testing:

Rubella performance was assessed with the CDC low titer sample (21 IU/mL). The sample was aliquoted, diluted in duplicate and tested by three technicians. Percent recovery was calculated for both neat and diluted samples.

CDC Low Titer Rubella Standard: 21 IU/mL						
Tech	Neat	Interpretation	% Recovery	1:2 Dilution	Interpretation	% Recovery
1	22	Positive	102%	11	Positive	102%
1	20	Positive	93%	10	Positive	99%
2	22	Positive	103%	11	Positive	109%
2	22	Positive	105%	11	Positive	104%
3	20	Positive	95%	11	Positive	101%
3	21	Positive	100%	12	Positive	111%

4. Clinical cut-off:

See section (1f).

5. Expected values/Reference range:

Observed prevalence was evaluated at three sites in a prospective study including individuals and pregnant women undergoing ToRCH testing.

1. 651 masked samples prospectively collected from individuals between the ages of <1 and 89 were tested at two external sites. 300 samples were submitted for ToRCH antibody assessment. 351 samples were submitted for testing of one or more of the analytes in the ToRCH panel. Testing was performed on all five markers on all samples. Results from a subset of 596/651 individuals between the ages of 17 and 69 were used to calculate HSV 1 and HSV 2 prevalence. Site 1, a hospital laboratory located in the Mid-Atlantic region tested 300 samples. Site 2, a hospital laboratory in the Northeast tested 351 samples.

2. 200 masked samples prospectively collected from pregnant women for

ToRCH antibody assessment were obtained from two serum vendors. The women ranged in age from 15 to 46. The samples were tested internally at the manufacturer site for all five analytes.

AtheNA Multi-Lyte ToRCH IgG Plus Observed Prevalence in Intended Use Populations

Prevalence of Analytes in Prospective Samples							
Age	Gender	Toxoplasma		Rubella		CMV	
		Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
0-9	Male	0/2	0.0%	1/2	50.0%	1/2	50.0%
	Female	1/2	50.0%	1/2	50.0%	1/2	50.0%
10-19	Male	1/3	33.3%	3/3	100.0%	2/3	66.7%
	Female	5/55	9.1%	50/55	90.9%	38/55	69.1%
20-29	Male	2/24	8.3%	23/24	95.8%	9/24	37.5%
	Female	58/257	22.6%	232/257	90.3%	184/257	71.6%
30-39	Male	3/16	18.8%	12/16	75.0%	8/16	50.0%
	Female	57/189	30.2%	170/189	89.9%	145/189	76.7%
40-49	Male	6/11	54.5%	11/11	100.0%	8/11	72.7%
	Female	14/44	31.8%	39/44	88.6%	30/44	68.2%
50-59	Male	1/10	10.0%	9/10	90.0%	5/10	50.0%
	Female	3/11	27.3%	10/11	90.9%	10/11	90.9%
60-69	Male	3/6	50.0%	6/6	100.0%	4/6	66.7%
	Female	3/4	75.0%	4/4	100.0%	3/4	75.0%
70+	Male	0/0	0.0%	0/0	0.0%	0/0	0.0%
	Female	1/1	100.0%	1/1	100.0%	1/1	100.0%
Unknown Age	Male	0/0	0.0%	0/0	0.0%	0/0	0.0%
	Female	2/13	15.4%	9/13	69.2%	10/13	76.9%
Unknown Gender/Age		0/3	0.0%	1/3	33.3%	3/3	100.0%
<b>Total</b>		160/651	24.6%	582/651	89.4%	459	71.0%

Prevalence of Analytes in Pregnant Women										
Age	Toxoplasma		Rubella		CMV		HSV 1		HSV 2	
	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence	Pos/Total	% Prevalence
16-19	2/23	9.2%	23/23	100.0%	16/23	69.6%	15/23	65.2%	3/23	13.0%
20-29	12/131	27.0%	35/39	89.7%	104/131	79.4%	92/124	74.2%	51/124	41.1%
30-39	10/37	55.6%	127/129	28.4%	26/37	70.3%	30/36	83.3%	14/36	38.9%
40-49	5/9	8.3%	9/9	100.0%	5/9	55.6%	8/9	88.9%	7/9	77.8%
<b>Total</b>	29/200	14.50%	194/200	97.90%	151/200	75.50%	160/200	80.00%	75/200	37.50%

**Observed Prevalence of HSV1 and HSV2 IgG in Sexually Active Adults**

		HSV 1		HSV 2	
Age	Gender	Pos/Total	% Prevalence	Pos/Total	% Prevalence
17-19	Male	0/0	0.0%	0/0	0.0%
	Female	27/38	71.1%	6/38	15.8%
20-29	Male	6/23	26.1%	1/23	4.3%
	Female	180/246	73.2%	57/245	23.3%
30-39	Male	10/16	62.5%	3/15	20.0%
	Female	165/187	88.2%	52/187	27.8%
40-49	Male	10/11	90.9%	3/11	27.3%
	Female	31/44	70.5%	16/44	36.4%
50-59	Male	5/10	50.0%	5/10	50.0%
	Female	8/11	72.7%	6/11	54.5%
60-69	Male	4/6	66.7%	2/6	33.3%
	Female	4/4	100.0%	2/4	50.0%
<b>Total</b>		450/596	75.5%	153/594	25.8%

**HSV Hypothetical Predictive Values by Prevalence**

Prevalence	Sexually Active Adults				Expectant Mothers			
	HSV-1		HSV-2		HSV-1		HSV-2	
	PPV	NPV	PPV	NPV	PPV	NPV	PPV	NPV
50.0%	94.8%	98.5%	93.7%	96.8%	87.0%	99.2%	92.9%	97.0%
40.0%	92.4%	99.0%	90.9%	97.8%	81.7%	99.5%	89.7%	98.0%
30.0%	88.7%	99.4%	86.5%	98.6%	74.2%	99.6%	84.9%	98.7%
25.0%	85.9%	99.5%	83.2%	98.9%	69.1%	99.7%	81.4%	99.0%
20.0%	82.0%	99.6%	78.8%	99.2%	62.6%	99.8%	76.6%	99.2%
15.0%	76.3%	99.7%	72.5%	99.4%	54.2%	99.9%	69.8%	99.5%
10.0%	67.0%	99.8%	62.4%	99.6%	42.7%	99.9%	59.3%	99.7%
5.0%	49.0%	99.9%	44.0%	99.8%	26.1%	100.0%	40.8%	99.8%

**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.