

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

A. 510(k) Number:

K063186

B. Purpose for Submission:

Clearance of new device

C. Measurand:

Rubella-specific IgG in serum

D. Type of Test:

Microparticle sandwich immunoassay

E. Applicant:

Zeus Scientific, Inc.

F. Proprietary and Established Names:

AtheNA Multi-Lyte Rubella IgG Test System

Rubella Virus Serological Reagents

G. Regulatory Information:

1. Regulation section:

21CFR §866.3510, Rubella virus serological reagents

2. Classification:

Class II

3. Product code:

LSD

4. Panel:

H. Intended Use:

1. Intended use(s):

The Zeus Scientific, Inc. AtheNA Multi-Lyte Rubella IgG Test System is intended for the qualitative detection of IgG class antibody to Rubella virus in human sera by microparticle immunoassay testing on the AtheNA Multi-Lyte instrument. The results of the AtheNA Multi-Lyte Rubella IgG Test System may be used to determine the serological status of individuals including women of childbearing age.

This test has been calibrated to the WHO International Standard for Rubella IgG at the cut-off. The magnitude of the test result above or below the cut-off does not correspond to International Units and is not indicative of total amount of antibody present.

2. Indication(s) for use:

The AtheNA Multi-Lyte Rubella IgG Test System aids in assessment of immune status to Rubella virus in human serum by detecting IgG antibodies to Rubella.

3. Special conditions for use statement(s):

For professional use

4. Special instrument requirements:

AtheNA Multi-Lyte instrument and software (cleared K042416)

I. Device Description:

The Zeus Scientific, Inc. AtheNA Multi-Lyte Rubella IgG test kit is a test system to detect IgG antibodies to the rubella virus in human serum, as an indicator of immunity to infection. The test is a microsphere bead-based sandwich immunoassay that detects IgG antibodies in serum, by means of binding of IgG in a specimen to antigen immobilized on microspheres in solution. The bound IgG is detected by a secondary anti-human IgG labeled with phycoerythrin. When analyzed on the AtheNA Multi-Lyte instrument, the bound phycoerythrin generates a luminescent signal in the presence of substrate, and the signal is proportional to the amount of IgG bound to the microspheres. The assay is calibrated by means of three calibration bead sets (cleared with this submission) whose values are optimized according to a process designed to yield a linear relationship between the fluorescence obtained and the unit values assigned. The calibration values self-adjust based on the unique characteristics

of the patient or control serum. The test results are reported as a unitless number derived from dividing the system readout by a correction factor to yield a number that is calibrated to the WHO International standard at the cut-off. This number differs from the International Units (IU) commonly used to report rubella IgG assay results. Values are reported with an associated interpretation of “negative”, “indeterminate”, or “positive” for the presence of anti-rubella IgG with a cut-off value of 10. The test results are to be used in conjunction with other clinical information and history to suggest immune status versus rubella virus.

Note: All descriptive information in the review summary below references AtheNA Multi-Lyte Assay results reported in AtheNA Units/mL. These units are not the final readout of the test system, and use a different scale from the final readout.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Zeus Scientific Inc., Rubella IgG ELISA Test System

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

K891783

3. Comparison with predicate:

Similarities and Differences		
Item	Device	Predicate
Intended Use	The Zeus Scientific, Inc. AtheNA Multi-Lyte Rubella IgG Test System is intended for the qualitative detection of IgG class antibody to Rubella virus in human sera by microparticle immunoassay testing on the AtheNA Multi-Lyte instrument. The results of the AtheNA Multi-Lyte Rubella IgG Test System may be used to determine the serological status of individuals including women of	The Zeus Scientific, Inc. Rubella ELISA Test System is designed for the qualitative and/or quantitative detection of IgG antibodies to rubella virus in human serum. The test system is intended to be used to evaluate single sera for immune status or paired sera to demonstrate seroconversion, and is for in vitro diagnostic use.

Similarities and Differences		
Item	Device	Predicate
	<p>childbearing age.</p> <p>This test has been calibrated to the WHO International Standard for Rubella IgG at the cut-off. The magnitude of the test result above or below the cut-off does not correspond to International Units and is not indicative of total amount of antibody present.</p>	
Test type	Qualitative	Qualitative/quantitative
Basic principle	Bead-based immunoassay	Solid phase immunoassay
Tracer	phycoerythrin, fluorescent signal	HRP-enzyme-labeled, colorimetric signal
Instrumentation	AtheNA Multi-Lyte instrument	ELISA microwell reader w/ wavelength = 450 nm, optional automated microwell wash system
Sample Type	Serum	Serum
Antigen Virus Strain	HPV-77	Unknown
Calibrator Format	Bead-based Intra-Well, calculated	Liquid, standard
Linearity with W.H.O. 1st International standard-Assay range	Yes, ~1-25 IU/mL	Yes, ~0-20 IU/mL
CDC Rubella Panel evaluation	Yes	No
Calibrators referenced to W.H.O.	No	Unknown
Secondary Antibody	Goat anti-human IgG; gamma chain specific	Goat anti-human IgG; gamma chain specific
Sample Volume	10 μ L of 1:21 dilution	100 μ L of 1:21 dilution
Calibrator Levels	4, values vary per lot	One
Reportable Range	<10, >10	0-20 IU/mL
Incubation time and temperature	30 \pm 5 min, 20-25° C	25 \pm 5 min, 20-25° C

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

NCCLS I/L6-A, “Detection and Quantitation of Rubella IgG Antibody: Evaluation and Performance Criteria for Multiple Component Test Products, Specimen Handling, and Use of the Test Products in the Clinical Laboratory”.

L. Test Principle:

The Zeus Scientific, Inc. AtheNA Multi-Lyte Rubella IgG Test System is designed to detect IgG class antibodies to Rubella in human sera. The test uses a sandwich immunoassay method. The diluted test sera are incubated in a vessel containing a multiplexed mixture of bead suspension. The multiplexed bead suspension contains a set of distinguishable sets of polystyrene microspheres. Conjugated to the primary set of microspheres is the Rubella 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. If present in patient sera, antibodies to the Rubella antigen will bind to the immobilized antigen on the primary bead set. The microspheres are rinsed to remove non-reactive serum proteins.

Phycoerythrin-conjugated goat anti-human IgG (Fc specific) is added to the vessel and the plate is incubated. The conjugate will react with the IgG antibody immobilized on the solid phase in step 1. The bead suspension is then analyzed by the AtheNA MultuLyte instrument. The bead set(s) are sorted, identified, and the amount of reporter molecule (PE conjugate) is determined for each bead set. Using proprietary *Intra-Well Calibration Technology*, internal calibration bead sets are used to evaluate unknown specimens to determine their immune status to rubella virus.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Precision was evaluated at three sites, using reagent lots and six samples, two each at 3 analyte levels on three instruments (one at each site). Each sample was evaluated 8 times per run, one run per day, for 3 days.

Intra-assay precision

		Units = AtheNA U/mL									
Sample		Day 1			Day 2			Day 3			N
		Mean	SD	CV (%)	Mean	SD	CV (%)	Mean	SD	CV (%)	
Site 1	1	655.5	30.6	4.7	660.8	29.7	4.5	677.3	29.5	4.4	24
	2	817.3	26.4	3.2	817.0	44.6	5.5	808.9	42.9	5.3	24
	3	2.9	2.4	84.1	3.5	2.7	77.9	2.9	1.7	60.1	24
	4	17.5	2.8	16.2	17.9	3.6	20.2	19.6	4.2	21.3	24

	5	159.4	6.9	4.3	154.6	15.4	10.0	157.6	12.7	8.0	24
	6	187.8	8.1	4.3	180.6	12.7	7.0	181.9	7.6	4.2	24
Site 2	1	653.9	15.7	2.4	602.1	35.4	5.9	646.9	24.9	3.9	24
	2	771.1	39.7	5.1	776.4	30.4	3.9	825.4	46.6	5.6	24
	3	6.0	3.6	60.4	3.9	2.5	65.3	6.0	1.8	29.5	24
	4	19.5	3.3	17.1	17.8	4.1	23.1	23.9	3.3	13.7	24
	5	154.5	8.4	5.4	149.8	12.8	8.6	163.9	12.6	7.7	24
	6	197.0	17.7	9.0	185.5	14.8	8.0	186.4	10.8	5.8	24
Site 3	1	643.8	30.9	4.8	648.6	33.6	5.2	682.6	23.6	3.5	24
	2	818.5	43.8	5.3	781.4	29.3	3.8	822.5	37.5	4.6	24
	3	6.6	3.3	50.3	4.6	2.6	55.4	5.9	3.7	62.7	24
	4	22.3	4.3	19.5	24.9	6.6	26.7	25.6	2.5	9.8	24
	5	166.8	6.3	3.8	168.3	8.4	5.0	178.6	8.1	4.6	24
	6	195.9	7.4	3.8	190.4	8.1	4.3	201.1	12.0	6.0	24

Inter-assay precision: precision across all assays per site

	Sample	Mean	SD	%CV
Site 1	1	664.5	30.1	4.5
	2	814.4	37.3	4.6
	3	3.1	2.2	72.8
	4	18.3	3.5	19.3
	5	157.2	11.8	7.5
	6	183.4	9.8	5.4
Site 2	1	634.3	34.5	5.4
	2	791.0	45.2	5.7
	3	5.3	2.8	53.3
	4	20.4	4.3	21.2
	5	156.0	12.5	8.0
	6	189.6	15.0	7.9
Site 3	1	658.3	33.4	5.1
	2	807.5	40.4	5.0
	3	5.7	3.2	56.0
	4	24.3	4.8	19.9
	5	171.2	9.1	5.3
	6	195.8	10.0	5.1

Between site summary: Overall precision

Sample	Mean	SD	%CV
1	652.4	34.8	5.3
2	804.3	41.7	5.2
3	4.7	3.0	63.4
4	21.0	4.9	23.2
5	161.5	13.1	8.1

6	189.6	12.8	6.7
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Assay reproducibility was determined according to CLSI I/L6-A, using a total of 93 samples; 47 “negative (<10 IU/mL) and 46 “positive” (10-20 IU/mL) as determined using an ELISA assay. Samples were tested in duplicate and the qualitative results were compared. The AtheNA Multi-Lyte Rubella IgG assay result (negative or positive) showed 100% agreement in results for all samples. Two samples with values near the cut-off of 10 IU/mL (120 AU/mL) were assayed forty times each.

	Sample 1	Sample 2
IU/mL	9.4	9.6
Mean AU/mL	144.4	134.0
Std deviation	10.1	12.0
CV	0.07	0.09

Lot-to-lot reproducibility was examined using 3 lots of reagent and 5 samples (2 positive, 2 negative, and one near the cut-off), each tested in quadruplicate. Intra- and inter-lot precision were calculated for each sample.

		Intra-Lot Precision			Inter Lot Precision	
		Lot 1	Lot 2	Lot 3		
Sample 94	mean	582.6	488.4	527.0	mean	532.7
	sd	56.0	25.7	46.0	sd	58.0
	%CV	9.6%	5.3%	8.7%	%CV	10.9%
Sample 53	mean	658.8	572.4	657.8	mean	629.6
	sd	29.5	37.2	38.8	sd	53.4
	%CV	4.5%	6.5%	5.9%	%CV	8.5%
Sample 22	mean	8.4	7.3	7.0	mean	7.5
	sd	2.3	1.8	1.7	sd	2.0
	%CV	27.8%	25.3%	24.1%	%CV	26.2%
Sample 32	mean	6.8	4.9	4.3	mean	5.3
	sd	1.4	0.8	2.0	sd	1.8
	%CV	20.6%	17.1%	46.6%	%CV	33.7%
Sample 83	mean	233.3	187.4	201.6	mean	207.4
	sd	12.8	9.1	11.4	sd	22.3
	%CV	5.5%	4.9%	5.6%	%CV	10.8%

b. *Linearity/assay reportable range:*

The AtheNA Multi-Lyte Rubella IgG assay reports results in “Athena Units (AU)/mL. These units do not correspond to WHO International Units

(IU)/mL.

The assay range is nominally 0-600 AU/mL. Assay linearity was defined with reference to the WHO Rubella IgG standard. Two-fold dilutions of the WHO standard were tested in singlicate using the AtheNA assay. Regression analysis of these results with the calculated WHO rubella IgG concentration yields the equation over the WHO concentration of 3.125-50 IU/mL (corresponding to 56-328 AU/mL):

$$y = 6.7347x + 34.101; R^2 = 0.9976$$

An additional linearity study was performed with additional dilutions of the WHO standard (1.56-25 IU/mL) with two lots of AtheNA reagent. Regression analysis over this range yields:

$$\text{Lot A: } R^2 = 0.9545$$

$$\text{Lot B: } R^2 = 0.9571$$

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

Calibration of the AtheNA Multi-Lyte Rubella IgG assay is by a proprietary software-driven interpretation of calibrators that have been value-assigned to generate a desired linear performance relationship between fluorescence obtained and the unit value(s) assigned. Unit values are based on the desired amount of activity in known positive and negative specimens. The manufacturer does not claim traceability to any reference material.

A review of 390 samples from the clinical population showed that 364/390 (93.3%) of individuals (age 10-70+, 21 males, 345 females) were positive for Rubella IgG by this assay.

Kit stability: Kit stability was determined on one lot of the test kit at 0, 8 and 14 months, using single determinations of positive and negative controls. Additional data was generated at 0 and 14 months using single determinations of seven samples covering the approximate measuring range of the assay. No deterioration of the test kit components was noted over this period.

d. Detection limit:

The detection limit of the assay is nominally 0 AU/mL. Four samples negative for Rubella IgG (as measured by another test system) were measured 20 times a day over 3 days. The mean limit of detection calculated was 8 AU/mL.

e. Analytical specificity:

Analytical specificity was demonstrated by testing of cross-reactivity with specimens containing IgG antibody to potentially cross-reacting subgroups.

Cross-reacting subgroups: 20 patient samples that were negative for rubella IgG were tested for presence of IgG antibodies to the following: VZV, mumps, measles, toxoplasmosis, CMV, VCA (EBV), HSV-1, and HSV-2. At least 5 of the 20 samples tested were positive for any one potentially cross-reacting IgG. Most samples test were positive for several pathogen-specific IgGs.

AtheNA Rubella IgG	IgG							
	VZV	Mumps	Measles	Toxo	CMV	EBV	HSV-1	HSV-2
14	0.72	4.16	2.03	0.14	0.14	3.56	3.53	0.77
16	1.39	1.51	0.36	0.12	1.95	4.00	3.77	1.33
16	1.51	6.39	1.81	0.06	1.92	3.36	5.47	2.48
12	1.09	3.99	5.17	0.05	2.19	0.07	0.10	0.06
16	0.92	2.06	2.14	0.17	1.78	3.43	3.54	1.12
8	2.59	0.95	4.68	0.35	2.82	2.56	0.19	0.09
4	1.79	5.33	3.25	0.10	2.57	1.17	4.64	0.99
8	0.89	7.75	3.00	0.02	2.06	2.69	0.41	0.13
9	1.64	4.26	2.60	0.08	2.12	0.78	3.86	0.89
9	0.71	1.11	0.37	0.08	2.64	3.60	1.39	0.59
5	0.74	1.83	0.48	0.12	0.10	3.20	0.12	0.06
11	1.47	0.39	0.88	3.53	1.95	1.16	2.40	0.52
10	2.33	2.97	1.45	0.20	1.72	4.07	4.21	0.96
10	1.32	2.33	0.27	1.35	1.21	3.35	3.49	0.88
12	1.36	4.39	2.10	0.25	0.69	4.59	3.56	0.64
17	2.69	1.43	2.46	2.92	2.69	3.55	4.32	1.44
6	0.41	1.65	5.61	0.09	4.03	4.98	0.22	0.17
6	2.34	0.44	0.11	2.42	0.16	2.50	3.76	1.10
6	1.12	1.58	0.97	6.17	2.95	1.76	3.84	0.75
7	2.59	6.73	4.57	0.03	0.07	5.21	0.12	0.17

Interpretation:

AtheNA Rubella (AU/mL)

1-99 = Negative

100-120 = equivocal

>120 = positive

All IgG ELISA assays (index value)

<= 0.90 = negative

0.91-1.09 = equivocal

>1.10 = positive

Interfering substances: Seven interfering substances (bilirubin, albumin, IgG,

cholesterol, triglycerides, hemoglobin, and intralipid) were examined for their effects on Rubella IgG measurement using 3 samples (positive, equivocal, negative) and two levels of spike of each interfering substance. PBS was used as a control to account for dilution in the unspiked samples.

Levels of interfering substance, mg/mL:

	Bilirubin	Albumin	IgG	Cholesterol	Triglyc	Hb	Intralipid
High	0.19	45	18	2	1.5	180	3.5
Low	0.36	90	36	4	3.0	360	7.0

		Percent of control signal recovered		
		Sample 1 (530 AU/mL)	Sample 2 (134 AU/mL)	Sample 3 (18 AU/mL)
Bilirubin	0.19 mg/mL	107.7	84.3	155.6
	0.36 mg/mL	90.4	78.4	138.9
Albumin	45 mg/mL	95.3	89.6	200.0
	90 mg/mL	114.0	73.1	183.3
IgG	18 mg/mL	126.8	320.1	2744.4
	36 mg/mL	134.5	403.0	2355.6
Cholesterol	2 mg/mL	107.4	94.0	166.7
	4 mg/mL	105.3	88.8	138.9
Triglycerides	1.5 mg/mL	101.5	84.3	172.2
	3.0 mg/mL	84.9	79.1	161.1
Hemoglobin	180 mg/mL	86.6	88.1	150.0
	360 mg/mL	96.4	72.4	155.6
Intralipid	3.5 mg/mL	94.9	86.6	144.4
	7.0 mg/mL	98.7	104.5	155.6

High levels of exogenous IgG caused systematic elevated rubella IgG results in the linear/near-linear range of the assay.

Bilirubin, triglycerides, and hemoglobin generally mildly suppressed rubella IgG values.

f. Assay cut-off:

The assay is calibrated to yield a cut-off value for the presence of anti-rubella IgG at 120 AU/mL, corresponding to approximately 10 IU/mL, by comparison to the WHO reference material. Fourteen dilutions of the WHO 1st International Rubella IgG standard (nominally 1600 IU/mL) were made, yielding calculated IgG concentrations of 0.0975-800 IU/mL. Each dilution

was measured in triplicate in two different kit lots of reagent. Results between 0.39-25 IU/mL were analyzed using linear regression

Linear regression	Lot 1	Lot 2
R square	0.9774	0.9798
Intercept	26.966	29.948
Slope	7.3288	8.5272
AtheNA result at WHO cut-off (10 IU/mL)	100.63 AU/mL	115.66 AU/mL

2. Comparison studies:

a. *Method comparison with predicate device:*

The AtheNA Multi-Lyte Rubella IgG assay was compared to the Zeus Scientific, Inc. Rubella IgG ELISA assay over the claimed working ranges of the assays, using 393 prospectively collected samples, at three sites (2 US, 1 Europe). The population tested was 372 females, 21 males, median age of 34 years, with 5 pediatric samples, and 346 women of childbearing age.

By site:

	Site 1	Site 2	Site 3
Positive agreement	81/82 (98.8%)	176/177 (99.4%)	110/113 (98.2)
Negative agreement	15/15 (100%)	1/1 (100%)	5/6 (100%)
Total agreement	96/97 (99.0%)	177/178 (99.4%)	115/118 (97.5)

All sites combined:

	All sites	95% CI
Positive agreement	367/371 (98.9%)	97.3-99.7
Negative agreement	21/21 (100%)	83.9-100
Total agreement	388/393 (98.7%)	97.1-99.6

Equivocal specimens in either assay were classified as “non-agreement”.

b. *Matrix comparison:*

N/A. Serum is only acceptable matrix

3. Clinical studies:

a. *Clinical Sensitivity:*

N/A

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

N/A

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.