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

A. 510(k) Number:

K102283

B. Purpose for Submission:

To obtain a substantial equivalence determination for the *Treponema pallidum* IgG ELISA test system

C. Measurand:

IgG antibodies to *Treponema pallidum*

D. Type of Test:

Enzyme linked immunosorbent assay

E. Applicant

Zeus Scientific Inc.

F. Proprietary and Established Names:

Treponema pallidum IgG ELISA Test System

G. Regulatory Information:

1. Regulation section:

   21 CFR § 866.3830, *Treponema pallidum* treponemal test reagents

2. Classification:

   Class II

3. Product code:

   LIP – Enzyme linked immunoabsorption assay, Treponema pallidum

4. Panel:

   83 - Microbiology

H. Intended Use:

1. Intended use:

   The ZEUS ELISA *Treponema pallidum* IgG Test System is intended for the qualitative detection of specific IgG class antibodies to *T. pallidum* in human serum. The test may be used in conjunction with non treponemal testing and clinical findings to provide serological evidence of infection with *T. pallidum.*

   This test is for *in vitro* diagnostic use only.

   This test is not intended for screening blood or plasma donors.

2. Indications for use:

   The ZEUS ELISA *Treponema pallidum* IgG Test System is intended for the qualitative detection of specific IgG class antibodies to *T. pallidum* in human serum. The test may be used in conjunction with non treponemal testing and clinical findings to provide serological evidence of infection with *T. pallidum.*

   This test is for *in vitro* diagnostic use only.

   This test is not intended for screening blood or plasma donors.

3. Special conditions for use statement:

   For Prescription Use

4. Special instrument requirements:

   ELISA microwell reader capable of reading at a wavelength of 450 nm

I. Device Description:

The Zeus Scientific Treponema pallidum ELISA Test System is intended for the qualitative detection of specific IgG class antibodies to T. pallidum in human serum. Each kit contains components in sufficient quantities to perform the number of tests indicated on the packaging label. The components are as follows: a 96 well plate configured in twelve 1x8 well strips coated with inactivated p 17 *T. pallidum* antigen; one 15 mL ready to use vial of conjugated (horse radish peroxidase) goat anti-human IgG (Fc chain specific); one 0.35 mL vial of positive control (human serum); one 0.5 mL vial of calibrator (human serum); one 0.35mL vial of negative control (human serum); SAVe diluent (sample diluent), ready to use, consisting of one 30 mL bottle containing Tween 20, bovine serum albumin and phosphate buffered saline, (pH 7.2 ±

0.2) ; one 15 mL bottle, ready to use, containing 3,3´,5,5´, tetramethylbenzidine (TMB), one ready to use 15 mL bottle of stop solution containing 1M H_2SO_4 and 0.7M HCL and wash buffer concentrate

J. Substantial Equivalence Information:

1. Predicate device name:

   Phoenix Bio-Tech Syphilis Trep-Chek Test

2. Predicate K number:

   K001552

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The Zeus Scientific Inc. *Treponema pallidum* ELISA Test System is intended for the qualitative detection of specific IgG class antibodies to *T. pallidum* in human serum. This test is for *in vitro* diagnostic use only. This test is not intended for screening blood or plasma donors, except for incubating or early primary disease.	The Phoenix Bio-Tech Corp. Syphilis Trep-Chek Test Kit is a confirmatory immunoassay for the qualitative detection of Treponema pallidum IgG antibodies in human serum or plasma. This product is not cleared (approved) by the U.S. Food and Drug Administration (FDA) for use in screening blood or plasma donors.
Assay type	Enzyme labeled immunoassay	Enzyme labeled immunoassay
Detection method	Colorimetric	Colorimetric
Matrix	Human serum	Human serum
Analyte	Human IgG antibodies	Human IgG antibodies
Conjugate label	Horse radish peroxidase	Horse radish peroxidase
Cut offs	Negative is <= 0.90, Positive is >= 1.10 and Equivocal is 0.90 - 1.09	Negative is <= 0.90, Positive is >= 1.10 and Equivocal is 0.90 - 1.09

Differences		
Item	Device	Predicate
Sample dilution	1:21 in Save diluent	1:20 in phophate buffer based diluent
Conjugate	Goat anti-human IgG; Fc chain specific	Goat anti-human IgG; γ chain specific
Sample & conjugate incubation	25+/- 5 minutes at room temperature	30+/- 2 minutes at room temperature

K. Standard/Guidance Document Referenced:

N/A

L. Test Principle:

The Zeus Scientific Inc. Treponema pallidum IgG ELISA Test System is designed to detect IgG antibodies to T. pallidum in human sera. Wells of plastic microwell strips are sensitized by passive absorption with T. pallidum antigen. The test procedure involves 3 incubation steps. Test sera are diluted with SAVe diluent . During sample incubation, any antigen specific IgG antibody in the sample will bind to the immobilized antigen. The plate is washed to remove unbound antibody and other serum components. Then peroxidase conjugated goat anti-human IgG is added to the wells and the plate is incubated. The conjugate will react with IgG antibody immobilized on the solid phase in step 1. The wells are washed to remove unbound conjugate. The microwells containing immobilized peroxidase conjugate are incubated with peroxidase substrate solution. Hydrolysis of the substrate by peroxidase produces a color change. After the incubation has ended, the reaction is stopped and the color intensity of the solution is measured photometrically. Color intensity of the solution depends on antibody concentration in the original test sample.

M. Performance Characteristics :

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

   a. *Precision/Reproducibility:*
   Precision was evaluated internally at the manufacturer's site. The study was conducted as follows: Fifteen samples, three for each category (negative, high negative, near cut-off, low positive and high positive) were identified and/or prepared (by Zeus Scientific, Inc.) for use in the study. To assess precision, on each day of testing, each sample was diluted twice and tested. This was repeated in a second run on the same day by a different technologist for a total of twelve days. Precision is considered acceptable for the reactive samples if

the total CV is <15%. and for the negative samples if the total CV is <25%. This study is summarized below:

Summary Of In-House Repeatability										
Panel	Sample	Mean	Within-Run		Within -Day		Between-Run		Total	
Member	N	AU/mL	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative 1	48	0.08	0.003	3.8%	0.006	8.0%	0.006	6.8%	0.011	13.1%
Negative 2	48	0.12	0.005	4.0%	0.003	6.5%	0.009	7.0%	0.013	10.5%
Negative 3	48	0.50	0.017	3.6%	0.034	9.5%	0.020	3.9%	0.045	9.0%
High Negative 1	48	0.75	0.057	7.6%	0.030	8.6%	0.019	2.5%	0.058	7.7%
High Negative 2	48	0.72	0.046	6.4%	0.014	7.1%	0.015	2.0%	0.052	7.2%
High Negative 3	48	0.74	0.015	2.0%	0.018	4.7%	0.011	1.4%	0.034	4.5%
Near Cut-off 1	48	0.92	0.028	3.0%	0.025	5.0%	0.036	3.9%	0.056	6.1%
Near Cut-off 2	48	1.04	0.022	2.1%	0.014	3.9%	0.033	3.1%	0.045	4.3%
Near Cut-off 3	48	0.95	0.037	3.9%	0.025	6.4%	0.036	3.8%	0.061	6.4%
Low Positive 1	48	1.48	0.029	2.0%	0.029	3.9%	0.014	0.9%	0.058	3.9%
Low Positive 2	48	1.43	0.026	1.8%	0.020	2.5%	0.017	1.2%	0.050	3.5%
Low Positive 3	48	1.65	0.027	1.6%	0.037	4.2%	0.018	1.1%	0.078	4.7%
High Positive 1	48	5.43	0.131	2.4%	0.154	3.8%	0.27	5.0%	0.38	7.0%
High Positive 2	48	4.85	0.110	2.3%	0.176	3.6%	0.17	3.6%	0.29	6.0%
High Positive 3	48	4.74	0.136	2.8%	0.189	4.9%	0.17	3.5%	4.74	5.2%
Non-Reactive Control	48	0.13	0.004	3.3%	0.008	6.23%	0.007	5.39%	0.010	8.2%
Reactive Control 1	48	5.63	0.049	7.5%	5.5	5.15%	0.29	5.11%	0.42	7.51%

Reproducibility was evaluated internally and at two external clinical sites. Fifteen samples, three for each category (negative, high negative, near cut-off, low positive and high positive) were identified and/or prepared for use in the study based upon their activity on the Treponema pallidum IgG ELISA assay. To assess reproducibility, on each day of testing, each sample was diluted twice and each dilution was run in triplicate. This process was repeated in a second run by a second technologist resulting in twelve results per day. This was repeated for five days at each site and the resulting data used to assess reproducibility. Reproducibility is considered acceptable for the reactive samples if the total CV is <15% and if the total CV for the border line and positive samples do not congregate at the high end of acceptability but show a spread of results at least 3%. The reproducibility for the negative sample is considered acceptable if the total CV is <50% and shows no change in the qualitative outcome. The study is summarized in the following table .

Summary Of Multi-Site Reproducibility												
Panel	Sample	Mean	Within-Run		Within -Day		Between-Run		Between-Site		Total	
Member	N	Index value	SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
Negative 1	180	0.06	0.01	14.7%	0.01	19.3%	0.01	10.7%	0.02	29.1%	0.02	36.6%
Negative 2	180	0.08	0.01	9.7%	0.01	12.2%	0.01	8.3%	0.01	14.6%	0.01	15.5%
Negative 3	180	0.31	0.03	8.6%	0.03	10.7%	0.02	6.6%	0.04	12.7%	0.04	13.3%
High Negative 1	180	0.80	0.04	5.1%	0.05	6.3%	0.03	4.2%	0.06	7.3%	0.06	7.3%
High Negative 2	180	0.74	0.04	5.1%	0.04	5.8%	0.02	3.0%	0.05	7.0%	0.05	7.4%
High Negative 3	180	0.76	0.04	5.0%	0.04	5.6%	0.21	2.7%	0.05	6.5%	0.05	7.2%
Borderline 1	180	1.05	0.07	6.3%	0.07	7.3%	0.03	3.6%	0.09	9.0%	0.11	10.3%
Borderline 2	180	1.13	0.05	4.7%	0.06	5.4%	0.04	3.1%	0.07	6.0%	0.08	6.7%
Borderline 3	180	0.95	0.05	5.6%	0.07	6.7%	0.04	4.0%	0.08	8.5%	0.09	9.9%
Low Positive 1	180	1.45	0.09	6.2%	0.11	7.6%	0.06	4.4%	0.13	8.9%	0.14	9.6%
Low Positive 2	180	1.77	0.11	5.9%	0.14	7.8%	0.10	5.8%	0.15	8.3%	0.16	9.2%
Low Positive 3	180	1.93	0.14	7.1%	0.17	8.9%	0.12	5.9%	0.19	9.7%	0.21	10.7%
Positive	180	3.6	0.20	5.7%	0.22	6.2%	0.10	2.8%	0.30	8.4%	0.37	10.2%
Positive 2	180	3.1	0.20	6.2%	0.22	7.3%	0.13	4.4%	0.28	9.0%	0.34	10.9%
Positive 3	180	3.1	0.18	5.7%	0.22	6.9%	0.16	4.9%	0.26	8.4%	0.31	10.2%
Non-Reac Control	180	0.09	0.01	10.6	0.01	12.8	0.01	6.2	0.01	15.9	0.02	21.0%
Reac Control	180	3.9	0.16	4.1	0.12	5.1	0.14	3.5	0.22	5.7	0.02	5.9

b. Linearity/assay reportable range:

Linearity for the T. pallidum test was assessed around the cut off. The WHO Syphilis Standard was diluted serially. Each dilution was tested in duplicate, the mean calculated and the result plotted. The linearity was acceptable.

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

N/A

d. Detection limit:

N/A

e. Analytical specificity:

Interference Testing

The effect of potential interfering substances on sample results generated using the test system was evaluated with the following possible interfering substances: albumin, bilirubin, cholesterol, hemoglobin, triglycerides and intralipids. Three samples (one positive for T. pallidum IgG, one borderline and one negative) were tested.

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)

The samples were exposed to the possible interfering substances and tested. An increase or reduction of signal equal to or less than 20% is considered acceptable. The negative sample may have a signal change greater than 20% if there is no change in the qualitative result of the sample.
All positive samples showed less than a 20% recovery of signal.
The borderline samples showed a recovery of signal <20% of with the exception of the high spike of hemoglobin (25.2%).
The negative sample showed a change of signal (>20%) with the high and low spikes of albumin, hemoglobin, intralipid, bilirubin, cholesterol and triglycerides. The negative sample results in each instance stayed below the cut-off and the change in signal did not affect the qualitative result.

Cross-Reactivity Study

Studies were performed to assess cross reactivity with T. pallidum IgG ELISA test system. Ten samples were tested for each cross-reactant. The results presented were obtained by testing the analytes against high concentrations of possible cross reactants. The results of this study are summarized in the following table. No cross reactivity was observed.

Treponema pallidum IgG Cross reactivity Study	
Analyte	positive/tested
EBV	0/10
ANA	0/10
RF IgM	0/10
Rubella	0/10
HIV	0/10
HSV 1	0/10
HSV 2	0/10
Sera from pregnant patients	0/10
Hepatitis B	0/10
VZV	0/10
VZV IgM	0/10
CMV	0/10
Toxoplasma	0/10
Lyme G/M	0/10
Hepatitis C	0/10

f. Assay cut-off:

N/A

2. Comparison studies:

a. Method comparison with predicate device:

N/A

b. Matrix comparison:

N/A

3. Clinical studies:

a. Clinical Sensitivity:

Five hundred unselected samples from patients with a syphilis test ordered as well as five hundred samples from pregnant women with a syphilis test ordered were submitted for syphilis antibody testing. They were sequentially numbered, de-identified and archived. These samples were tested at Zeus, a hospital lab in the Mid-Atlantic and another in the Northeast. Results are listed in the following tables:

		Banked sera from patients with syphilis test ordered					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA	Positive	4	0	3	7	80.0%	28.4-99.5%
	Equivocal	1	0	1	2		
	Negative	0	0	491	491	99.2%	97.9-99.8%
	Site Total	5	0	495	500		

		Banked purchased sera from pregnant women with syphilis test ordered					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA							
	Positive	3	0	0	3	75.0%	19.4-99.4%
	Equivocal	0	0	0	0		
	Negative	1	0	494	495	100.0%	99.4-100%
	Site Total	4	0	494	498		

*2 samples excluded due to unverifiable age

Additional clinical performance was assessed by testing samples from 1000 hospitalized patients at the three clinical sites.

		Unselected hospitalized patients					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA							
	Positive	13	1	18	32	61.9%	38.4-81.9%
	Equivocal	1	0	9	10		
	Negative	7	0	950	957	97.1%	95.9-98.1%
	QNS	0	0	1	1		
	Site Total	21	1	978	1000		

A total of 223 banked known positive HIV-1 samples were tested with the device.

		Banked purchased known HIV-1 positive serum samples					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA							
	Positive	41	0	1	42	85.4%	72.2-93.9%
	Equivocal	1	0	0	1		
	Negative	4	2	174	180	99.4%	96.9-100%
	Site Total	46	2	175	223		

280 samples requested to be RPR/TPPA positive were purchased from a vendor and were tested. Additionally, 250 negative samples and 27 RPR/TPPA positive samples from pregnant women were also tested with the device. Results are listed in the tables below:

		Banked purchased sera requested to be RPR/TPPA reactive					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA							
	Positive	259	1	4	264	98.5%	96.2-99.6%
	Equivocal	1			1		
	Negative	3		12	15	70.6%	46.9-98.7%
	Site Total	263	1	16	280		

		Banked purchased sera from pregnant women requested to be TPPA positive (27) RPR/TPPA non-reactive (250)					
		Predicate					
		Positive	Equivocal	Negative	Site Total	PPA NPA	95% CI
T. pallidum IgG ELISA							
	Positive	26	1	0	27	92.9%	76.5-99.1%
	Equivocal	0	0	0	0		
	Negative	2	0	248	250	99.6%	97.8-100%
	Site Total	28	1	248	277		

The CDC syphilis panel consisting of a total of 157 clinically characterized samples was also tested. Samples were from patients with varying stages of syphilis. The results from the T. pallidum IgG test system were compared to the CDC panel.

Clinical Diagnosis	T. pallidum IgG ELISA Results				% Agreement with Clinical Diagnosis Presented with 95% CI	
	Positive	Equivocal	Negative	Total		
Primary Treated	11	0	0	11	100% (11/11)	76.2-100%
Secondary Untreated	41	0	2	43	95.3% (41/43)	84.2-99.4%
Secondary Treated	39	0	0	39	100% (39/39)	92.6-100%
Latent Untreated	6	0	5	11	54.5% (6/11)	23.4-83.3%
Latent Treated	48	0	2	50	96.0% (48/50)	86.3-99.5%
Congenital	1	1	1	3	33.3% (1/3)	0.84-90.6%
Total	146	1	10	157	93.0% (146/157)	87.8-96.5%

*b. Clinical specificity**:***

See Sec. M.3.a

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

N/A

4. Clinical cut-off**:**

N/A

5. Expected values/Reference range:

Random unselected sera submitted for syphilis antibody testing were tested with the device, to determine expected values in the populations tested. In the 500 prospectively collected samples from patients ranging in age from <1 to >70 years of age, 7 tested positive. The overall observed prevalence in this group was 1.4% (7/500 samples).
In the 500 samples collected from pregnant women ranging in age from 15 to 48, 3/498 samples tested positive. The observed prevalence in this group was 0.6%. Two samples were excluded due to questionable age.
In the group of 1000 samples from unselected hospitalized patients ranging in age from <1 to >70 years of age, 32 tested positive. The overall observed prevalence in this group was 3.2% (32/999 samples). One sample was QNS for testing.

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.