510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K231616

B Applicant

ZEUS Scientific

C Proprietary and Established Names

ZEUS IFA nDNA Test System
ZEUS dIFine

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
KTL	Class II	21 CFR 866.5100 - Antinuclear Antibody Immunological Test System	IM - Immunology
PIV	Class II	21 CFR 866.4750 - Automated indirect immunofluorescence microscope and software-assisted system	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Migration of previously cleared assay to a previously cleared instrument

B Measurand:

Anti-double stranded DNA (dsDNA) IgG autoantibodies

C Type of Test:

Qualitative and/or semi-quantitative indirect immunofluorescence (IFA); manual or semi-automated

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

The ZEUS IFA nDNA Test System is an indirect immunofluorescence assay utilizing Crithidia luciliae for the qualitative and semi-quantitative determination of anti-native DNA (nDNA) IgG antibodies to DNA in human serum by manual fluorescence microscopy or with ZEUS dIFine. The presence of nDNA antibodies in conjunction with other serological and clinical findings can be used to aid in the diagnosis of systemic lupus erythematosus (SLE).

C Special Conditions for Use Statement(s):

Rx - For Prescription Use Only

- This device is only for use with reagents that are indicated for use with the device.
- The device is for use by a trained operator in a clinical laboratory setting.
- All software-aided results must be confirmed by a trained operator.

D Special Instrument Requirements:

For use only with Zeus dIFine

IV Device/System Characteristics:

A Device Description:

ZEUS IFA nDNA Test System is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-nativeDNA (nDNA) IgG antibodies in human serum by manual fluorescence microscopy or with ZEUS dIFine.

<u>ZEUS IFA nDNA Test System Assay kit components</u>

1. *C. luciliae* Substrate Slides: Twenty, 10-well Slides with blotter.
2. Conjugate: Goat anti-human IgG labeled with FITC. Contains phosphate buffer with BSA and counterstain. One bottle (white-capped amber bottle), 12 mL. Ready to use.
3. Positive Control (Human Serum): Will produce positive apple-green staining of the kinetoplast in the *C. luciliae* organisms. One vial (red-capped), 0.5 mL. Ready to use.
4. Negative Control (Human Serum): Will produce no detectable nDNA staining. One vial (green-capped), 0.5 mL. Ready to use.
5. SAVe Diluent: Four bottles (green-capped), 30 mL phosphate-buffered-saline. Ready to use.
6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Ten packets, each sufficient to prepare one liter PBS in distilled or deionized water.
7. Mounting Media (Buffered Glycerol): One bottle (clear-capped clear bottle), 12 mL. Ready to use.

B Principle of Operation:

The ZEUS IFA nDNA Test System is an indirect fluorescent antibody assay for the qualitative and semi-quantitative determination of anti-nDNA IgG antibody in human sera by manual fluorescence microscopy or by ZEUS dIFine. The reaction occurs in two steps:

1. Step one: If nDNA antibodies are present in a sample, a reaction between nDNA antibodies and the kinetoplast of the *C. luciliae* substrate takes place in the first step.
2. Step two: Goat anti-human IgG labeled with fluorescein isothiocyanate (FITC) is added to the substrate. If the patient's sera contain anti-nDNA IgG antibody, a positive apple-green, fluorescent antigen-antibody reaction will be observed when the slides are examined with the fluorescence microscope. Smooth or peripheral staining of the kinetoplast with a bright fluorescence located near the flagellar region of the *C. luciliae* is considered a positive reaction.

Manual Interpretation: The interpretation of the results depends on the pattern observed as well as the titer of the autoantibody present in the specimen. A positive reaction is the presence of any pattern of nuclear apple-green staining observed at a 1:10 dilution based on a 1+ to 4+ scale of staining intensity where 1+ is considered a weak reaction and 4+ a strong reaction. The sponsor recommends sera positive at 1:10 should be titered to endpoint dilution by making 1:20, 1:40, 1:80, etc. serial dilutions. The endpoint titer is the highest dilution that produces a 1+ positive reaction.

Zeus dIFine Interpretation: When slides are analyzed by Zeus dIFine, digital images of representative fields of view of the well are captured. The default scanning area is composed of 12 fields with each field approximately 610 µm x 510 µm using the 20X objective. All images are taken through a fluorescein isothiocyanate (FITC) filter. The ZEUS dIFine System reads the slides and measures the FITC light intensity of the *Crithidia* that are included in the region. Then, the ZEUS dIFine System reports the measured average nuclear fluorescence intensity as an index percentage and recommends a qualitative result. To facilitate the interpretation, the ZEUS dIFine System presents acquired digital images of the slide and the following information for human analysis: Negative, Positive or Uncertain (only for Zeus IFA nDNA Test System on ZEUS dIFine with automated reading (software interpretation) of the slides) classification

Trained operators then review the images taken by ZEUS dIFine System. During the review process, further options include:

- Navigation of digitized well using virtual microscope tools (zooming/browsing images at different magnifications).
- Enlargement of images to examine detail.
- Image Atlas to assist with identification of patterns.

The trained operator can confirm the results by clicking the "Validate" button on the screen and accepting the classification (negative/positive and pattern) suggested by the ZEUS dIFine System, or they can revise the suggested ZEUS dIFine classification (negative to positive and vice versa or from Uncertain to either positive or negative), add comments (if any) and eventually click the "Validate" button on the screen.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Test System For nDNA Antibody Determination

B Predicate 510(k) Number(s):

K780178

C Comparison with Predicate(s):

Device & Predicate Device(s):	K231616	K780178
Device Trade Name	Zeus IFA nDNA Test System, Zeus dIFine	Zeus IFA nDNA Test System
General Device Characteristic Similarities		
Intended Use/ Indications for Use	The ZEUS IFA nDNA Test System is an indirect immunofluorescence assay utilizing *Crithidia luciliae* for the qualitative and semi quantitative determination of anti-native DNA (nDNA) IgG antibodies to DNA in human serum by manual fluorescence microscopy or with ZEUS dIFine. The presence of nDNA antibodies in conjunction with other serological and clinical findings can be used to aid in the diagnosis of systemic lupus erythematosus (SLE).	This is an indirect fluorescent antibody test for the semi-quantitative detection of IgG anti-nDNA antibodies in human serum. This test system is to be used as an aid in the diagnosis of systemic lupus erythematosus.
Methodology	Indirect immunofluorescence assay	Same
Sample Matrix	Serum	Same
Fluorescence Marker	FITC	Same
Control	One positive control and one negative control	Same
Screening Dilution	1:10	Same
Antigen	*Crithidia luciliae*	Same
Results	Qualitative, semi-quantitative	Same
Storage Conditions	Unopened test system, mounting media, Conjugate Zorba-NS, Slides, Positive control, and Negative control must be stored at 2-8 ºC	Same
General Device Characteristic Differences		
Interpretation of results	Manual fluorescence microscopy or Zeus dIFine automated microscopy with trained operator verification	Manual fluorescence microscopy

VI Standards/Guidance Documents Referenced:

The following Clinical and Laboratory Standards Institute (CLSI) guidelines were used:

- CLSI EP05-A3, Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline – Third Edition
- CLSI EP06-Ed2, Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A3, Interference Testing in Clinical Chemistry – Third Edition
- CLSI EP17-A2, Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures – Second Edition
- CLSI EP28-A3c, Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory

VII Performance Characteristics (if/when applicable):

All analytical and clinical studies were evaluated by comparing the three possible reading methods (A, B, and C) described in the table below; these methods are consistent throughout this document. Method A (i.e., manual imaging and manual reading of the slides with a traditional fluorescence microscope) is considered the reference method (predicate) to which all results are compared. All results generated by the Zeus IFA nDNA Test System must be confirmed by a trained operator.

Table 1. Interpretation Methods

Method	Processing	Imaging	Reading/Evaluation of Slides
A (predicate)	Manual	Manual	Manual (read of microscope field)
B	Automated	Automated	Manual (read of digital image)
C	Automated	Automated	Automated (software interpretation)

A Analytical Performance:

1. Precision/Reproducibility:

   a. Repeatability - 20-Day Within-Laboratory Precision Study

   Two low positive serum samples (~1:10/1:20 endpoint), two medium positive serum sample (~1:40 to 1:80 endpoint), two high positive serum samples (>1:160 endpoint) and two negative specimens were assayed in triplicate, on 20 different days producing 60 results per sample at one site. The slides were interpreted by all three methods for qualitative results. All slides were interpreted via Methods A and B independently by two technicians, as well as Method C using a single instrument.

Within-Method Qualitative Result Agreement:

There was 100% within-method qualitative result agreement with the expected result for all eight samples when interpreted via Methods A and B, for both technicians. For Method C, the medium positive sample#1, high positive sample#2, and both negative samples yielded 100% within-method qualitative result agreement. The low positive sample#1, low positive sample#2, medium positive sample#2, and high positive sample#1 yielded within-method qualitative result agreement values of 96.7%, 98.3%, 98.3%, and 95.0%, respectively. The results are summarized in the following tables:

Table 2: Within-Method Qualitative Result Agreement

Sample	**Agreement (95% CI)**		
	Method A	**Method B**	**Method C**
Low Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	96.7% (88.6 - 99.1%)
Low Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)
High Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	95.0% (86.3 - 98.3%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)

Between-Method Agreement:

There was 100% between-method qualitative result agreement for all eight samples when interpreted via Method A versus Method B, for both technicians. When Method A and Method B were compared to Method C, the medium positive sample# 1, high positive sample#2, and both negative samples yielded 100% between-method qualitative result agreement. The low positive sample#1, low positive sample#2, medium positive sample#2, and high positive sample#1 yielded between-method qualitative result agreement values of 96.7%, 98.3%, 98.3%, and 95.0%, respectively. The results are summarized in the following tables:

Table 3: Between-method Qualitative Agreement

Sample	Agreement (95% CI)		
	Method A vs Method B	**Method A vs Method C**	**Method B vs Method C**
Low Positive-1	100% (88.7 - 100%)	96.7% (88.6 - 99.1%)	96.7% (88.6 - 99.1%)
Low Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
Medium Positive-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Medium Positive-2	100% (88.7 - 100%)	98.3% (91.1 - 99.7%)	98.3% (91.1 - 99.7%)
High Positive-1	100% (88.7 - 100%)	95.0% (86.3 - 98.3%)	95.0% (86.3 - 98.3%)
High Positive-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-1	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)
Negative-2	100% (88.7 - 100%)	100% (88.7 - 100%)	100% (88.7 - 100%)

b. Reproducibility – 5-day Site-to-Site Reproducibility Study

Two negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (>1:320 endpoint) were assayed at a 1:10 screening dilution in triplicate, twice per day, on five different days, at three different laboratories. Qualitative results were interpreted by two technicians at each laboratory for Methods A and B, and by a single dIFine instrument at each laboratory for Method C. The results are summarized in the following tables.

Table 4: Qualitative Agreement for Method A between Technicians Across Three Sites:

Method A		**Site 1**		**Site 2**		**Site 3**	
		Technician 1	**Technician 2**	**Technician 1**	**Technician 2**	**Technician 1**	**Technician 2**
Site 1	**Technician 1**		100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)
	Technician 2			100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)
Site 2	**Technician 1**				100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)
	Technician 2					100% (98.42-100.00)	100% (98.42-100.00)
Site 3	**Technician 1**						100% (98.42-100.00)
	Technician 2						

Table 5: Qualitative Agreement for Method B between Technicians Across Three Sites:

Method B		Site 1		Site 2		Site 3	
		Technician 1	Technician 2	Technician 1	Technician 2	Technician 1	Technician 2
Site 1	Technician 1		100% (98.42-100.00)	100% (98.42-100.00)	100% (98.42-100.00)	99.17% (97.01-99.77)	99.17% (97.01-99.77)
	Technician 2			100% (98.42-100.00)	100% (98.42-100.00)	99.17% (97.01-99.77)	99.17% (97.01-99.77)
Site 2	Technician 1				100% (98.42-100.00)	99.17% (97.01-99.77)	99.17% (97.01-99.77)
	Technician 2					99.17% (97.01-99.77)	99.17% (97.01-99.77)
Site 3	Technician 1						100% (98.42-100.00)
	Technician 2						

Table 6: Qualitative Agreement for Method C across Three Sites:

Method C	Site 1	Site 2	Site 3
Site 1		94.58% (90.95 - 96.81)	95.83% (92.50 - 97.72)
Site 2			96.25% (93.03 - 98.01)
Site 3			

c. Lot-to-Lot Reproducibility

Nine negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (> 1:320 endpoint) were assayed at a 1:10 screening dilution using three different reagent lots. For the six positive samples, additional serial dilutions ranging from 1:20 through 1:5120, were also assayed and interpreted by all three methods, towards determining an endpoint titer. There was 100% agreement in the qualitative results at the screening dilution for all 15 specimens across all three kit lots, for interpretation methods A and B. For lot 3, one UNC result was obtained via interpretation method C at the 1:10 screening dilution, for one of the low positive samples. All six positive specimens resulted in the same endpoint titers ± one dilution regardless of reagent kit lot or method interpretation.

2. Linearity:

Two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (>1:320 endpoint) were assayed at a 1:10 screening dilution, as well as at serial dilutions ranging from 1:20 through 1:5120, then interpreted by all three methods. Consistent positivity was found throughout dilution to endpoint and a consistent titer endpoint was found between methods (within ±1 titer). Individual results per dilution were read reported using a fluorescence intensity scale

from “0” (negative) to “4” (high positive). The endpoints for each sample and each method are presented below:

Table 7: Endpoint Titers across Methods

Sample	Method A	Method B	Method C
Low Positive-1	1:10	1:20	1:20
Low Positive-2	1:20	1:40	1:40
Medium Positive-1	1:40	1:80	1:80
Medium Positive-2	1:40	1:80	1:80
High Positive-1	1:640	1:640	1:640
High Positive-2	1:640	1:640	1:640

Table 8: The sample dilutions and associated intensity grade results for Method A

Sample	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Low Positive-1	1	0	0	0	0	NT	NT
Low Positive-2	2	1	0	0	0	NT	NT
Medium Positive-1	2	1	1	0	0	0	0
Medium Positive-2	2	1	1	0	0	0	0
High Positive-1	4	3	3	2	2	1	1
High Positive-2	4	3	3	2	2	1	1

Table 9: The sample dilutions and associated intensity grade results for Method B

Sample	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Low Positive-1	1	1	0	0	0	NT	NT
Low Positive-2	2	1	1	0	0	NT	NT
Medium Positive-1	2	2	1	1	0	0	0
Medium Positive-2	2	2	1	1	0	0	0
High Positive-1	4	3	3	2	2	1	1
High Positive-2	4	3	3	2	2	1	1

Method C cannot provide an intensity grade, only a positive, negative, or uncertain call.

3. Analytical Specificity/Interference:

   a. Interference studies

   Two negative serum samples, two low positive serum samples (~1:10-1:20 endpoint), two medium positive serum samples (~1:40-1:80 endpoint), and two strong positive serum samples (> 1:320 endpoint) were spiked with two different concentrations (low spike and high spike) of the 19 different interferents outlined in the table below. All specimens were assayed in triplicate by the ZEUS IFA nDNA Test System and interpreted by all three methods. Qualitative results were interpreted by two technicians for Methods A and B, and by a single dIFine instrument for Method C.

Table 10: List of Endogenous and Exogenous Interferents

Endogenous Interfering Substance	Maximum Concentration
Hemoglobin	200 mg/mL
Bilirubin	0.15 mg/mL
Triglycerides	2.5 mg/mL
Cholesterol	2.2 mg/mL
Rheumatoid Factor	400 IU/mL
Intralipids	20 mg/mL
Albumin	52 mg/mL

Exogenous Interfering Substance	Maximum Concentration
Azathioprine	0.00258 mg/mL
Belimumab	8 mg/mL
Cyclophosphamide	0.549 mg/mL
Diltiazem	0.0009 mg/mL
Enalapril	0.000819 mg/mL
Hydroxychloroquine	0.24 mg/mL
Ibuprofen	0.219 mg/mL
Intralipids	20 mg/mL
Methotrexate	1.36 mg/mL
Mycophenolate Mofetil	0.048 mg/mL
Naproxen	0.36 mg/mL
Prednisone	0.000099 mg/mL
Rituximab	2 mg/mL
Simvastatin	0.000083 mg/mL
Voclosporin	0.00021 mg/mL

None of the interferents affected the expected results of any samples when read by Methods A and B. When the interferent/samples combinations were tested, Method C yielded uncertain results in several samples: 'low negative-2' sample spiked with a high concentration of cyclophosphamide, 'low positive-2' sample spiked with hydroxychloroquine, 'medium positive-2' sample spiked with a low concentration of azathioprine, and a high concentration of bilirubin, 'high positive-1' sample spiked with a high concentration of albumin, and a low concentration of triglycerides.

b. Cross-Reactivity

The analytical cross-reactivity of the assay was evaluated using 23 International Consensus on Antinuclear Antibody (ANA) Patterns (ICAP) reference samples tested at a 1:10 dilution with all three interpretation methods. The results of this study demonstrate that most of the ANA exhibited by members of the ICAP panel do not cross react with the kinetoplast of the Crithidia in the ZEUS IFA nDNA Test System except for ANA-1 (ANA Homogenous positive), ANA-7 (Anti SS-A Ro Positive), ANA-23 (Anti Rods/Rings positive) that were positive across all three interpretation methods.

4. Assay Reportable Range:

   Not applicable

5. Traceability, Stability, Expected Values (Controls, Calibrators, or Methods):

   a. Traceability

      A recognized standard or reference material for anti-dsDNA antibodies for immunofluorescence is not available.

   b. Stability

      i). Open Reagent Stability
      The slides must be used the same day as they are opened. All other ready-to-use reagents, except PBS, may be used until their stated expiration date.

      ii). Unopened Reagent Stability
      The reagents for this assay are the same as those in the predicate device. The real-time stability studies support shelf-life claim of 24 months when stored at 2–8ºC.

      iii). Specimen Stability
      The real-time sample stability data supports sample storage at room temperature (20–25ºC) for no longer than 8 hours. If testing is not performed within 8 hours, sera may be stored between 2–8°C, for no longer than 48 hours. If delay in testing is anticipated, test sera must be stored at –20°C or lower.

6. Detection Limit:

   Not applicable

7. Assay Cut-Off:

   Please refer to K7810178. Zeus recommends a screening dilution of 1:10 and any titers less than 1:10 are considered negative.

B Comparison Studies:

1. Method Comparison with Predicate Device:

   The method comparison study was performed using clinical samples (described in Section C.1) at three independent laboratories in the U.S. Considering the interpretations recorded from all three sites for these 660 specimens, there were a total of 3,960 instances where the results of Method A versus Method B, Method A versus Method C, and Method B versus Method C were compared. Two technicians tested each sample at each site. Method A is considered as the predicate.

a. Method A vs Method B Agreement

Table 11: Qualitative Agreement between Method A and Method B for each Site and each Technician

Method A vs Method B		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 1	**Tech 1**	100.00% (83/83) (95.58 – 100.00)	100.00% (577/577) (99.34 – 100.00)	100.00% (660/660) (99.42 – 100.00)
	Tech 2	98.78% (81/82) (93.41 – 99.78)	99.65% (576/578) (98.75 – 99.91)	99.55% (657/660) (98.67 – 99.85)
Site 2	**Tech 1**	100.00% (74/74) (95.07 – 100.00)	99.66% (584/586) (98.76 – 99.91)	99.70% (658/660) (98.90 – 99.92)
	Tech 2	97.44% (76/78) (91.12 – 99.29)	100.00% (582/582) (99.34 – 100.00)	99.70% (658/660) (98.90 – 99.92)
Site 3	**Tech 1**	100.00% (80/80) (95.42 – 100.00)	99.31% (576/580) (98.24 – 99.73)	99.39% (656/660) (98.45 – 99.76)
	Tech 2	100.00% (80/80) (95.42 – 100.00)	98.97% (574/580) (97.76 – 99.53)	99.09% (654/660) (98.03 – 99.58)

Table 12: Combined Qualitative Agreement for all Sites/all Technicians

		Method A		Total
		Positive	**Negative**	
Method B	**Positive**	474	14	488
	Negative	3	3469	3472
	Total	477	3483	3960

PPA: 99.37% (474/477) (95% CI: 98.17% to 99.79%)
NPA: 99.60% (3469/3483) (95% CI: 99.33% to 99.76%)
Overall Agreement: 99.57% (3943/3960) (95% CI: 99.31% to 99.73%)

b. Method A vs Method C Qualitative Comparison

Since Method C can yield an uncertain (UNC) result in addition to a positive or negative qualitative result, the agreement between methods were calculated using the UNC samples considered positive and then considered negative:

Table 13: UNC considered as Positive for each Site and each Technician

Method A vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 1	**Tech 1**	97.59% (81/83) (91.63-99.34)	98.44% (568/577) (97.06 - 99.18)	98.33% (649/660) (97.04 - 99.07)
	Tech 2	95.18% (79/83) (88.25-98.11)	98.10% (567/578) (96.63 - 98.93)	97.88% (646/660) (96.47 - 98.73)
Site 2	**Tech 1**	98.65% (73/74) (92.73-99.76)	98.46% (577/586) (97.11 - 99.19)	98.48% (650/660) (97.23 - 99.17)
	Tech 2	97.44% (76/78) (91.13-99.29)	98.97% (576/582) (97.77 - 99.53)	98.79% (652/660) (97.63 - 99.38)

Method A vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 3	Tech 1	97.50% (78/80) (91.34-99.31)	98.45% (571/580) (97.08 - 99.18)	98.33% (649/660) (97.04 - 99.07)
	Tech 2	96.25% (77/80) (89.55-98.72)	98.28% (570/580) (96.86 - 99.06)	98.03% (647/660) (96.66 - 98.85)

Table 14: Combined Qualitative Agreement between Method A and Method C for all Sites/all Technicians (UNC as positives):

		Method A		Total
		Positive	Negative	
Method C	Positive	464	54	518
	Negative	13	3429	3442
	Total	467	3483	3960

PPA: 97.27% (464/467) (95% CI: 95.39% to 98.40%)
NPA: 98.45% (3429/3483) (95% CI: 97.98% to 98.81%)
Overall Agreement: 98.31% (3893/3960) (95% CI: 97.86% to 98.66%)

Table 15: UNC considered as Negative for each Site and each Technician

Method A vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 1	Tech 1	95.18% (79/83) (88.25 - 98.11)	99.13% (572/577) (97.99 – 99.63)	98.64% (651/660) (97.43 - 99.28)
	Tech 2	93.90% (77/82) (86.51 - 97.37)	98.79% (571/578) (97.52 - 99.41)	98.18% (648/660) (96.85 - 98.96)
Site 2	Tech 1	91.89% (68/74) (83.42 - 96.23)	99.83% (585/586) (99.04 - 99.97)	98.94% (653/660) (97.83 - 99.49)
	Tech 2	88.46% (69/78) (79.50 - 93.81)	100.00% (582/582) (99.34 - 100.00)	98.64% (651/660) (97.43 - 99.28)
Site 3	Tech 1	96.25% (77/80) (89.55 - 98.72)	100.00% (580/580) (99.34 - 100.00)	99.55% (657/660) (98.67 - 99.86)
	Tech 2	95.00% (76/80) (87.84 - 98.04)	99.83% (579/580) (99.03 - 99.97)	99.24% (655/660) (98.24 - 99.68)

Table 16: Combined Qualitative Agreement between Method A and Method C for all Sites/all Technicians (UNC as Negatives):

		Method A		Total
		Positive	Negative	
Method C	Positive	446	14	460
	Negative	31	3469	3500
	Total	477	3483	3960

PPA: 93.50 % (446/477) (95% CI: 90.92% to 95.38%)
NPA: 99.60% (3469/3483) (95% CI: 99.33% to 99.76%)
Overall Agreement: 98.31% (3893/3960) (95% CI: 97.86% to 98.66%)

c. Method B vs Method C Qualitative Comparison

Since Method C can yield an uncertain (UNC) result in addition to a positive or negative qualitative result, the agreement between methods were calculated using the UNC samples considered positive and then considered negative:

Table 17: UNC considered as Positive for each Site and each Technician

Method B vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 1	Tech 1	97.59% (81/83) (91.63 - 99.34)	98.44% (568/577) (97.06 - 99.18)	98.33% (649/660) (97.04 - 99.07)
	Tech 2	96.39% (80/83) (89.90 - 98.76)	98.27% (567/577) (96.84 - 99.06)	98.03% (647/660) (96.66 - 98.85)
Site 2	Tech 1	97.37% (74/76) (90.90 - 99.28)	98.63% (576/584) (97.32 - 99.30)	98.48% (650/660) (97.23 - 99.17)
	Tech 2	98.68% (75/76) (92.92 - 99.77)	98.80% (577/584) (97.55 - 99.42)	98.79% (652/660) (97.63 - 99.38)
Site 3	Tech 1	96.43% (81/84) (90.02 - 98.78)	98.96% (570/576) (97.75 - 99.52)	98.64% (651/660) (97.43 - 99.28)
	Tech 2	95.35% (82/86) (88.64 - 98.18)	99.13% (569/574) (97.98 - 99.63)	98.64% (651/660) (97.43 - 99.28)

Table 18: Combined Qualitative Agreement between Method B and Method C for all Sites/all Technicians (UNC as positives):

		Method B		Total
		Positive	Negative	
Method C	Positive	473	45	518
	Negative	15	3427	3442
	Total	488	3472	3960

PPA: 96.93 % (473/488) (95% CI: 94.99% to 98.13%)
NPA: 98.70 % (3427/3472) (95% CI: 98.27% to 99.03%)
Overall Agreement: 98.48% (3900/3960) (95% CI: 98.06% to 98.82%)

Table 19: UNC considered as Negative for each Site and each Technician

Method B vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 1	Tech 1	95.18% (79/83) (88.25 - 98.11)	99.13% (572/577) (97.99 - 99.63)	98.64% (651/660) (97.43 - 99.28)
	Tech 2	93.98% (78/83) (86.66 - 97.40)	98.96% (571/577) (97.75 - 99.52)	98.33% (649/660) (97.04 - 99.07)
Site 2	Tech 1	89.47% (68/76) (80.58 - 94.57)	99.83% (583/594) (99.04 - 99.97)	98.64% (651/660) (97.43 - 99.28)
	Tech 2	90.79% (69/76) (82.19 - 95.47)	100.00% (584/584) (99.35 - 100.00)	98.94% (653/660) (97.83 - 99.49)

Method B vs Method C		Positive Sample Agreement (n/N) (95% CI)	Negative Sample Agreement (n/N) (95% CI)	Total Sample Agreement (n/N) (95% CI)
Site 3	Tech 1	91.67% (77/84) (83.78 - 95.90)	100.00% (576/576) (99.34 - 100.00)	98.94% (653/660) (97.83 - 99.49)
	Tech 2	89.53% (77/86) (81.29 - 94.40)	100.00% (574/574) (99.34 - 100.00)	98.64% (651/660) (97.43 - 99.28)

Table 20: Combined Qualitative Agreement between Method A and Method C for all Sites/all Technicians (UNC as Negatives):

		Method B		Total
		Positive	Negative	
Method C	Positive	448	12	460
	Negative	40	3460	3500
	Total	488	3472	3960

PPA: 91.80 % (448/488) (95% CI: 89.03% to 93.92%)
NPA: 99.65% (3460/3472) (95% CI: 99.40% to 99.80%)
Overall Agreement: 98.69% (3908/3960) (95% CI: 98.28% to 98.82%)

2. Matrix Comparison:

Not applicable

C Clinical Studies:

1. Clinical Sensitivity and Clinical Specificity:

Clinically characterized specimens, described below, were tested to determine the clinical sensitivity and specificity of the assay on all three methods. The samples were acquired from commercial sources, aliquoted into three samples, then tested at three separate clinical laboratories. At all sites, the same cohort of 300 samples associated with Systemic Lupus Erythematosus (SLE) and 360 samples associated with non-SLE diseases (ANA-associated diseases and non-ANA associated diseases) were tested. The samples were tested at the screening 1:10 sample dilution then read by all three methods. At all three sites, Method A and Method B were interpreted by two different laboratory technicians.

Table 21: Clinically Characterized Samples used in the Study

Target Disease		***n***
Systemic Lupus Erythematosus		300
Control Diseases		***n***
ANA-Associated Diseases		
Connective Tissue Diseases	Sjögren's Syndrome	30
	Scleroderma	20
	Autoimmune Myositis	30
	Mixed Connective Tissue Disease	20
	CREST	20

Other ANA-Associated Autoimmune Diseases	Autoimmune Hepatitis	20
	Primary Biliary Cholangitis	10
	Drug-Induced Lupus	20
Non-ANA-Associated Diseases		
Other Autoimmune Diseases	Celiac	20
	Vasculitis (ANCA)	30
	Crohn's Disease	10
	Rheumatoid Arthritis	30
	Autoimmune Thyroiditis	30
	Inflammatory Bowel Disease	10
	Ulcerative Colitis	10
Other Diseases	Fibromyalgia	10
	Infectious Disease	20
	Malignancy/Cancer	20
Total:		**660**

The clinical sensitivity was calculated at each site on SLE while specificity was calculated using the ANA associated diseases and non-ANA-associated diseases described above. The clinical sensitivity and specificity for each site and each technician is presented in the table below:

Table 22: Clinical Performance at Site 1

Diagnostic Sensitivity and Specificity		**SLE**	**Control Diseases**
		% Sensitivity (95% CI)	**% Specificity (95% CI)**
Method A	**Technician A**	26.67 (21.98 - 31.94)	99.17 (97.58 - 99.72)
	Technician B	27.00 (22.29 - 32.29)	99.72 (98.44 - 99.95)
Method B	**Technician A**	26.67 (21.98 - 31.94)	99.17 (97.58 - 99.72)
	Technician B	27.00 (22.29 - 32.29)	99.44 (98.00 - 99.85)
Method C	**dIFine**	27.00 (22.29 - 32.29)	99.17 (97.58 - 99.72)

Table 23: Clinical Performance at Site 2

Diagnostic Sensitivity and Specificity		**SLE**	**Control Diseases**
		% Sensitivity (95% CI)	**% Specificity (95% CI)**
Method A	**Technician A**	24.33 (19.82 - 29.49)	99.72 (98.44 - 99.95)
	Technician B	25.00 (20.44 - 30.20)	99.17 (97.58 - 99.72)
Method B	**Technician A**	25.00 (20.44 - 30.20)	99.72 (98.44 - 99.95)
	Technician B	24.33 (19.82 - 29.49)	99.17 (97.58 - 99.72)
Method C	**dIFine**	22.33 (17.99 - 27.38)	99.17 (97.58 - 99.72)

Table 24: Clinical Performance at Site 3

Diagnostic Sensitivity and Specificity		SLE	Control Diseases
		% Sensitivity (95% CI)	% Specificity (95% CI)
Method A	Technician A	25.33 (20.74 - 30.55)	98.89 (97.18 - 99.57)
	Technician B	25.67 (21.05 - 30.90)	99.17 (97.58 - 99.72)
Method B	Technician A	25.33 (20.74 - 30.55)	97.78 (95.68 - 98.87)
	Technician B	25.67 (21.05 - 30.90)	97.50 (95.32 - 98.68)
Method C	dIFine	24.33 (19.82 - 29.49)	97.50 (95.32 - 98.68)

D Clinical Cut-Off:

Not applicable

E Expected Values/Reference Range:

One hundred and eighty serum samples were acquired from apparently healthy donors. Samples were collected from donors within the United States. Each sample was tested at the screening dilution of 1:10 using the ZEUS IFA nDNA Test System, then scanned by ZEUS dIFine and the qualitative results (i.e., Positive, Negative or Uncertain) were determined via Methods A, B, and C. The percent positivity was determined for the 1:10 screening data. The results for the reference range are summarized below in the table:

Table 25: Reference Range Determination (N=180)

Method	Number of Positives	% Positives	Number of Negatives	% Negatives	Number of Uncertain	% Uncertain
A	2	1.11%	178	98.89%	NA	NA
B	2	1.11%	178	98.89%	NA	NA
C	1	0.56%	176	97.78%	3	1.67%

VIII Proposed Labeling:

The labeling supports the finding of substantial equivalence for this device.

IX Conclusion:

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