510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY AND INSTRUMENT

I Background Information:

A 510(k) Number

K201956

B Applicant

Zeus Scientific, Inc.

C Proprietary and Established Names

Zeus IFA ANA HEp-2 Test System,
Zeus dIFine

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
DHN	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:

Assay and Instrument

B Measurand:

Anti-nuclear antibodies (ANA)

C Type of Test:

Qualitative and semi-quantitative, indirect immunofluorescence assay (IFA)

III Intended Use/Indications for Use:

A Intended Use(s):

See Indications for Use below.

B Indication(s) for Use:

Assay:

ZEUS IFA ANA HEp-2 Test System is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of IgG anti-nuclear antibodies in human serum by manual fluorescence microscopy or with ZEUS dIFine. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus and other systemic rheumatic diseases. All suggested results obtained with ZEUS dIFine must be confirmed by a trained operator.

Instrument:

ZEUS dIFine is an automated instrument consisting of a fluorescent microscope and software that acquires, interprets, stores and displays digital images of stained indirect immunofluorescence slides. ZEUS dIFine can only be used with FDA cleared or approved ZEUS in vitro diagnostic assays that are indicated for use on this instrument. All suggested results obtained with ZEUS dIFine must be confirmed by a trained operator.

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 the trained operator.

D Special Instrument Requirements:

For use only with Zeus dIFine

IV Device/System Characteristics:

A Device Description:

ZEUS IFA ANA HEp-2 Test System is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of IgG anti-nuclear antibodies (ANA) in human serum by manual fluorescence microscopy or with ZEUS dIFine.

ZEUS IFA ANA HEp-2 Test System Assay kit components

1. ANA Hep-2 Substrate slides: Twenty, 12-well slides with absorbent blotter and desiccant pouch.
2. Conjugate: Goat anti-human IgG labeled with fluorescein isothiocyanate (FITC). Contains phosphate buffer with BSA and counterstain. One bottle, 12 mL, white-capped. Ready to use.
3. Positive Control (Human Serum): Will produce positive apple-green, homogeneous, staining of the cell nucleus. One vial, 0.5 mL, red-capped. Ready to use.
4. Negative Control (Human Serum): Will produce no detectable nuclear staining. One vial, 0.5 mL, green-capped. Ready to use.
5. Zorba-NS Sample Diluent: Phosphate-buffered-saline. Four bottles, 30 mL, green-capped. Ready to use.
6. Phosphate-buffered-saline (PBS): pH 7.2 ± 0.2. Ten packets, each sufficient to prepare one liter.
7. Mounting Media (Buffered Glycerol): One clear bottle, 12 mL, clear-capped. Ready to use.

ZEUS dIFine

The ZEUS dIFine System is an automated fluorescence microscope. It does not process samples or perform the assay, but acquires digital images of representative areas of the ZEUS ANA HEp-2 Test System Assay (IFA) slides. The instrument hardware includes the microscope, camera, microscope control unit, and an LED illumination unit. The ZEUS dIFine System includes an Olympus fluorescence microscope with 4X and 20X objectives. The microscope has a motorized slide stage that accommodates up to eight slides. ZEUS dIFine System includes an industrial computer with Windows 10 operating system as well as dIFine software v 1.5.28.

B Principle of Operation:

ZEUS IFA ANA HEp-2 Test System is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of IgG anti-nuclear antibodies in human serum by manual fluorescence microscopy or by ZEUS dIFine. The ZEUS IFA ANA HEp-2 Test System detects the presence of circulating ANA in human sera that bind to HEp-2 cells coated on the glass slide. Goat anti-human immunoglobulin-FITC conjugate visualizes the ANA bound to the cells. Any positive reactions will appear as apple-green colored fluorescent staining within the cell. If the sample does not contain ANA, there will be no distinct staining of the cell.

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 1:40 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 1:40 should be titered to endpoint dilution by making 1:40, 1:80, 1:160, etc. serial dilutions. The endpoint titer is the highest dilution that produces a 1+ positive reaction.

Zeus dIFine Interpretation: When slides are analyzed by 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 cells 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, and a pattern if it is one of the eight patterns

recognized by the software which will be confirmed by a human reader. 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 ANA HEp-2 Test System on ZEUS dIFine with automated reading (software interpretation) of the slides) classification
- Positive Pattern Information: Zeus dIFine can suggest eight patterns - homogeneous, nucleolar, centromere, speckled, nuclear membrane, nuclear dots, cytoplasmic (ribosomal), cytoplasmic (mitochondrial).
- Image Atlas containing a gallery of mitotic figures (on positive samples of identified patterns).

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), add comments (if any) and eventually click the "Validate" button on the screen.

C Instrument Description Information:

1. Instrument Name:

   Zeus dIFine

2. Specimen Identification:

   Manual Entry or Integrated Barcode slide scanner

3. Specimen Sampling and Handling:

   Not applicable

4. Calibration:

   Not applicable

5. Quality Control:

   Not applicable.

V Substantial Equivalence Information:

A Predicate Device Name(s):

Antinuclear Antibody Test System Tissue Cell Culture Slide

B Predicate 510(k) Number(s):

K781516

C Comparison with Predicate(s):

Device & Predicate Device(s):	K201956	K781516
Device Trade Name	Zeus IFA ANA HEp-2 Test System, Zeus dIFine	Zeus IFA ANA HEp-2 Test System - ANA Test System Tissue Cell Culture Slide
General Device Characteristic Similarities		
Intended Use/ Indications For Use	ZEUS IFA ANA HEp-2 Test System is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of IgG anti-nuclear antibodies in human serum by manual fluorescence microscopy or with ZEUS dIFine. The presence of anti-nuclear antibodies can be used in conjunction with other serological tests and clinical findings to aid in the diagnosis of systemic lupus erythematosus and other systemic rheumatic diseases. All suggested results obtained with ZEUS dIFine must be confirmed by a trained operator.	The ZEUS IFA ANA HEp-2 Test System is a pre-standardized assay designed for the qualitative and semi-quantitative detection of antinuclear antibodies. The test is intended to aid in determining SLE and differentiating clinically similar connective tissue disorders and is for *In Vitro* diagnostic use.
Methodology	Indirect immunofluorescence assay	Same
Results	Pattern and titer Qualitative and semi-quantitative	Same
Sample Matrix	Serum	Same
Fluorescence Marker	FITC	Same
Control	One positive control and one negative control	Same
Screening Dilution	1:40	Same
Antigen	Hep-2 cells	Same

Slides	12-well coated with antigen	Same
Storage Conditions	Unopened test system, mounting media, Conjugate Zorba-NS, Slides, Positive control and Negative control must be stored at 2-8ºC	Same
Counterstain	Evans Blue	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:

- CLSI EP05-A3 Evaluation of Precision Performance of Quantitative Measurement Methods, Approved Guideline - 3rd Edition
- CLSI EP06, 2nd ed. Evaluation of the Linearity of Quantitative Measurement Procedures – Second Edition
- CLSI EP07-A2 Interference Testing in Clinical Chemistry, 2nd Edition
- CLSI EP17-A2 Evaluation of Detection Capability for Clinical Laboratory Measurement Procedures, 2nd Edition
- CLSI EP28-A3c Defining, Establishing and Verifying Reference Intervals in the Clinical Laboratory
- IEC 61010-1 Safety Requirements for Electrical Equipment for Measurement, Control, and Laboratory Use Part 1: General Requirements
- ISO 14971 - Medical Devices - Application of risk management to medical devices, Second Edition
- Guidance for Industry and FDA Staff - Guidance for the Content of Premarket submission for Software Contained in Medical Devices (May 11, 2005)

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 ANA HEp-2 Test System must be confirmed by a trained operator.

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)

The Zeus dIFine identifies the following immunofluorescence (IF) patterns: homogenous, speckled, centromere, nucleolar, nuclear dots, nuclear membrane, cytoplasmic (Ribosomal) and cytoplasmic (Mitochondrial).

A Analytical Performance:

1. Precision/Reproducibility:

a. Repeatability - 10-Day Within-Laboratory Precision Study

A low positive serum sample (~1:40 endpoint), a mid-positive serum sample (~1:160 to 1:320 endpoint) and a strong positive serum sample (>1:640 endpoint) were identified for each of the eight patterns reported by dIFine. One ANA negative specimen was also included bringing the group of specimens to a total of 25. These 25 specimens were diluted at 1:40 and assayed in triplicate, on ten different days producing 30 results per sample at one site and by one reader. The slides were interpreted by all three methods for qualitative results. The pattern was determined by all methods for all positive samples.

The study produced 30 results for each of the 25 specimens for each of the three interpretation methods (Method A, Method B and Method C). The results of the qualitative and pattern agreement for within-method and between-methods are evaluated.

For the within-method agreement based on qualitative results and pattern, there was 100% qualitative agreement in all samples when read using Methods A and B. For Method C, 22 out of 25 specimens showed 100% qualitative agreement; homogenous low positive, nucleolar low positive, and nuclear membrane low positive specimens did not have complete qualitative agreement. Additionally, there was 100% pattern agreement within Methods A and B. For method C, there was 100% pattern agreement for 21 out of 25 specimens with outliers observed in homogenous low positive, nucleolar low positive and nuclear membrane low positive and nuclear membrane high positive specimens. The results are summarized in the following tables:

Within-Method Qualitative Result Agreement			
Sample	**Method A Agreement (95% CI)**	**Method B Agreement (95% CI)**	**Method C Agreement (95% CI)**
Homogenous Low Positive	100% (88.7-100%)	100% (88.7-100%)	76.7% (59.1-88.2%)
Homogenous Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Homogenous High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Within-Method Qualitative Result Agreement			
Sample	**Method A Agreement (95% CI)**	**Method B Agreement (95% CI)**	**Method C Agreement (95% CI)**
Centromere Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar Low Positive	100% (88.7-100%)	100% (88.7-100%)	96.7% (83.3-99.4%)
Nucleolar Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondria Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane Low Positive	100% (88.7-100%)	100% (88.7-100%)	90% (74.4-96.5%)
Nuclear Membrane Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Negative	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Within-Method Pattern Result Agreement			
Sample	**Method A Agreement (95% CI)**	**Method B Agreement (95% CI)**	**Method C Agreement (95% CI)**
Homogenous Low Positive	100% (88.7-100%)	100% (88.7-100%)	73.3% (55.6-85.8)
Homogenous Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Homogenous High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar Low Positive	100% (88.7-100%)	100% (88.7-100%)	96.7% (83.3-99.4%)
Nucleolar Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondria Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane Low Positive	100% (88.7-100%)	100% (88.7-100%)	86.67% (70.3-94.7%)
Nuclear Membrane Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane High Positive	100% (88.7-100%)	100% (88.7-100%)	96.7% (83.3-99.4%)
Nuclear Dots Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Within-Method Pattern Result Agreement			
Sample	**Method A Agreement (95% CI)**	**Method B Agreement (95% CI)**	**Method C Agreement (95% CI)**
Ribosomal Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

For the between-method agreement based on qualitative results and pattern, there was 100% qualitative and pattern agreement between Method A versus Method B. However, only 22 out of 25 specimens showed 100% qualitative in the comparisons between Method B vs Method C and Method A vs Method C; the discrepant samples were the homogenous low positive, nucleolar low positive and nuclear membrane low positive specimens. Additionally, only 21 out of 25 specimens showed 100% pattern agreement in the comparisons between Method B vs Method C and Method A vs Method C; the discrepant samples were the homogenous low positive, nucleolar low positive and nuclear membrane low positive and nuclear membrane high positive specimens. The results are summarized in the following tables:

Between-Method Qualitative Result Agreement			
Sample	**Method A vs Method B Agreement (95% CI)**	**Method A vs Method C Agreement (95% CI)**	**Method B vs Method C Agreement (95% CI)**
Homogenous Low Positive	100% (88.7-100%)	76.67% (59.1-88.2%)	76.67% (59.1-88.2)
Homogenous Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Homogenous High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Between-Method Qualitative Result Agreement			
Sample	**Method A vs Method B Agreement (95% CI)**	**Method A vs Method C Agreement (95% CI)**	**Method B vs Method C Agreement (95% CI)**
Nucleolar Low Positive	100% (88.7-100%)	96.67% (83.3-99.4)	96.67% (83.3-99.4)
Nucleolar Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondria Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane Low Positive	100% (88.7-100%)	90% (74.4-96.5%)	90% (74.4-96.5%)
Nuclear Membrane Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Negative	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Between-Method Pattern Result Agreement			
Sample	**Method A vs Method B Agreement (95% CI)**	**Method A vs Method C Agreement (95% CI)**	**Method B vs Method C Agreement (95% CI)**
Homogenous Low Positive	100% (88.7-100%)	73.3% (55.6-85.9%)	73.3% (55.6-85.9%)
Homogenous Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Homogenous High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

Between-Method Pattern Result Agreement			
Sample	**Method A vs Method B Agreement (95% CI)**	**Method A vs Method C Agreement (95% CI)**	**Method B vs Method C Agreement (95% CI)**
Speckled Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Speckled High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Centromere High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar Low Positive	100% (88.7-100%)	96.7% (83.3-99.4%)	96.7% (83.3-99.4%)
Nucleolar Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nucleolar High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondria Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Mitochondrial High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane Low Positive	100% (88.7-100%)	86.7% (70.3-94.7%)	86.7% (70.3-94.7%)
Nuclear Membrane Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Membrane High Positive	100% (88.7-100%)	96.7% (83.3-99.4%)	96.7% (83.3-99.4%)
Nuclear Dots Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Nuclear Dots High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Low Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal Mid Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)
Ribosomal High Positive	100% (88.7-100%)	100% (88.7-100%)	100% (88.7-100%)

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

A low positive serum sample (~1:40 endpoint), a mid-positive serum sample (~1:160 to 1:320 endpoint) and a strong positive serum sample (> 1:640 endpoint) were identified for each of the eight ANA patterns reported by dIFine. One ANA negative specimen was also included bringing the group of specimens to a total of 25. These 25 specimens were assayed (1:40 screening dilution) in triplicate, two times per day on five different days at three different sites/laboratories, producing 30 results per sample, per location. At each site, Method A and Method B were also interpreted by two separate laboratory technicians. Therefore, for Method A and Method B, there were 30 assays of each of the 25 specimens but 60 interpretations of those 25 specimens. The slides were interpreted by all three methods for qualitative results and pattern determinations for all positive samples.

Qualitative Agreement:

Within Method:
Within method qualitative agreement for Method A and Method B was 100% for all three sites and all technicians. For Method C, there was 100% agreement for 24 out of 25 specimens at all sites and all technicians; one nuclear membrane low positive specimen was discrepant at Site 2.

Between Method:
There was 100% qualitative agreement for all 25 specimens between Method A versus Method B and Method A versus Method C. Notably, there was 100% qualitative agreement between Method B vs Method C for 24 out of 25 specimens with the nuclear membrane low positive specimen being discrepant at Site 2.

Pattern Agreement:

Within Method:
Within method pattern agreement for Method A and Method B was 100% for all three sites and all technicians. For Method C, there was 100% agreement for 24 out of 25 specimens with centromere mid specimen as the only discrepant result.

Between Method:
There was 100% pattern agreement for all 25 specimens between Method A vs B for all sites and all technicians. For Method A vs Method C, there were two discrepant samples: a centromere mid specimen at Site 1 and a nuclear membrane low specimen at Site 2. Similarly, for Method B vs Method C, there were two discrepant samples: a centromere mid specimen at Site 1 and a nuclear membrane low specimen at Site 2.

c. Lot-to-Lot Precision

The lot-to-lot precision of the qualitative and pattern results was evaluated in a 5-Day study using three lots of Zeus IFA ANA Hep-2 Test. A mid positive serum sample (~1:160 to 1:320 endpoint) and a strong positive sample (>1:640 endpoint) were identified for each of the eight ANA patterns. Nine ANA negative specimens were also included bringing the group of specimens to a total of 25 samples. The 16 positive

samples were serially titered through endpoint, assayed on three separate lots of Zeus IFA ANA Hep-2 Test system and then scanned by Zeus dIFine. The nine negative samples were assayed at a 1:40 screening dilution on three separate lots of Zeus IFA ANA Hep-2 Test system and then scanned by Zeus dIFine. The slides were interpreted by all three methods for qualitative results and pattern determinations.

Qualitative Agreement: There was 100% agreement in the qualitative results at the screening dilution of all 25 specimens across all three kit lots, and there was 100% agreement across all three interpretation methods regardless of reagent kit lot.

Endpoint Titer Agreement: All 16 positive specimens resulted in endpoint titers +/- one dilution regardless of reagent kit lot or method interpretation.

Pattern Agreement: For lot 1 and lot 2, there was 100% pattern agreement for each specimen by all three interpretation methods. For lot 3, there was 100% pattern agreement for each specimen between Method A and Method B, with one homogenous mid positive specimen as the only discrepant specimen when comparing Method C versus Methods A and B.

2. Linearity:

A strong positive serum sample was identified for each of the eight ANA patterns. Each of the samples was serially diluted from a 1:40 titer to 1:20,480 titer then qualitatively interpreted as positive or negative by all three methods as noted above. The endpoint titer was considered as the last positive well within a titration series. In general, the fluorescent intensity decreased (e.g., +4 to +1) with an increase in titer for the samples. Patterns did not change when the samples were diluted; the same patterns were recovered in every dilution until negative.

The endpoint titers across samples for each method for each of eight samples with eight ANA patterns are shown in the table below:

Endpoint Titers across Samples and Methods			
Sample	**Method A**	**Method B**	**Method C**
Homogenous	1:1280	1:1280	1:1280
Speckled	1:5120	1:5120	1:5120
Centromere	1:5120	1:5120	1:5120
Nucleolar	1:2560	1:5120	1:2560
Nuclear Dots	1:640	1:640	1:640
Nuclear Membrane	1:2560	1:2560	1:2560
Cytoplasmic (Ribosomal)	1:320	1:320	1:320
Cytoplasmic (Mitochondrial)	1:10240	1:10240	1:10240

In all samples the pattern called was as expected and in agreement regardless of the method of interpretation. Likewise, the endpoint determination was within one dilution for all of the determinations regardless of the method of interpretation.

3. Analytical Specificity/Interference:

a. Interference studies:

The interference study was performed according to CLSI EP07-A2 to determine the effect of various endogenous and exogenous substances on the ZEUS IFA ANA HEp-2 Test System in combination with ZEUS dIFine. A mid-positive sample (~1:160 to 1:320 endpoint) and a strong positive sample (≥1:640 endpoint) were identified for each of the eight ANA patterns. One ANA negative specimen was also included bringing the group of specimens to a total of 17. These 17 specimens were spiked with two different concentrations (low spike and high spike) of 12 different possible interferents as outlined in the table below. All specimens were evaluated on the ZEUS IFA ANA HEp-2 Test System and interpreted by all three methods. For methods A and B, neither the qualitative agreement nor the resulting pattern was affected by the addition of the possible interferents. Method C yielded one uncertain result in a low positive nuclear membrane sample when spiked with Albumin.

A second interference study was conducted using a low positive sample (approximate endpoint titer 1:40/1:80), for each of the eight ANA patterns along with one negative serum sample tested with some additional potential interferents. Exogenous and endogenous interferants were added at high and low concentrations to each of the serum samples listed. Each of the nine serum samples listed above were assayed at a 1:40 sample dilution using the ZEUS IFA ANA HEp-2 Test System, then scanned by ZEUS dIFine. Qualitative results (i.e., Positive or Negative) were determined via Methods A, B, and C for all samples.

Endogenous and Exogenous Interferants Tested			
Endogenous Interfering Substance	**Maximum Concentration**	**Exogenous Interfering Substance**	**Maximum Concentration**
Hemoglobin	200 mg/mL	Prednisone	0.000099 mg/mL
Bilirubin	0.15 mg/mL	Cyclophosphamide	0.549 mg/mL
Triglycerides	2.5 mg/mL	Ibuprofen	0.219 mg/mL
Cholesterol	2.2 mg/mL	Hydroxychloroquine	0.24 mg/mL
Rheumatoid Factor	400 IU/mL	Simvastatin	0.000083 mg/mL
Intralipids	20 mg/mL	Azathioprine	0.00258 mg/mL
Albumin	52 mg/mL	Diltiazem	0.0009 mg/mL
		Mycophenolate Mofetil	0.048 mg/mL
		Rituximab	2 mg/mL
		Belimumab	8 mg/mL

Neither the qualitative agreement nor the resulting pattern was affected by the addition of the possible interferents at the concentrations listed in the table, regardless of the method of interpretation.

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 1:40 dilution with all three interpretation methods. All qualitative results are in line with the characterization by ICAP and did not indicate cross-reactivity to ANA.

4. Assay Reportable Range:

Not applicable

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

a. Traceability

There is no recognized standard for anti-nuclear antibodies.

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 eight hours. If testing is not performed within eight 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 K781516. Zeus recommends a screening dilution of 1:40 and any titers less than 1:40 are considered negative.

8. Accuracy (Instrument):

Not applicable

9. Carry-Over:

Not applicable

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. At Sites 1 and 3, 392 samples were tested; at Site 2, 350 samples were tested. Two technicians tested each sample at each site. Method A is considered as the predicate.

a. **Qualitative comparisons**:

The qualitative agreement between the methods was determined by testing a 1:40 sample dilution of each sample with the ZEUS IFA ANA HEp-2 Test System then read using all three methods. Method A and Method B readings were read using two different laboratory technicians. The positive samples were titered to endpoint and the pattern was determined. Positive percent agreement (PPA) and negative percent agreement (NPA) values were calculated, with 95% confidence intervals.

i. Method A vs Method B Qualitative Comparison

Qualitative Agreement between Method A and Method B				
Method A vs Method B		**Positive Sample Agreement (95% CI)**	**Negative Sample Agreement (95% CI)**	**Total Sample Agreement (95% CI)**
Site 1	Tech 1	95.6% (152/159) (91.2-97.9%)	95.7% (223/233) (92.3-97.7%)	95.7% (375/392) (93.2-97.3%)
	Tech 2	94.9% (168/177) (90.6-97.3%)	98.6% (212/215) (95.9-99.5%)	96.9% (380/392) (94.7-98.2%)
Site 2	Tech 1	99.2% (124/125) (95.6-99.9%)	93.8% (211/225) (89.8-96.3%)	95.7% (335/350) (93.1-97.4%)
	Tech 2	98.6% (137/139) (94.9-99.6%)	97.6% (206/211) (94.6-98.9%)	98.0% (343/350) (95.9-99.0%)
Site 3	Tech 1	95.5% (148/155) (90.9-97.8%)	98.7% (234/237) (96.4-99.6%)	97.5% (382/392) (95.4-98.6%)
	Tech 2	95.9% (162/169) (91.7-97.9%)	97.8% (218/223) (94.9-99.0%)	96.9% (380/392) (94.7-98.2%)

Combined Qualitative Agreement for Method A and Method B				
		Method A		**Total**
		Positive	**Negative**	
Method B	**Positive**	891	40	931
	Negative	33	1304	1337
Total		924	1344	2268

PPA: 96.4% (891/924) (95% CI: 95.0%; 97.5%)
NPA: 97.0% (1304/1344) (95% CI: 96.0%; 97.8%)
Overall Agreement: 96.8% (2195/2268) (95% CI: 96.0%; 97.4%)

ii. 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:

Qualitative Agreement between Method A and Method C (When UNC from Method C Considered as Positive)				
Method A vs Method C		**Positive Sample Agreement (95% CI)**	**Negative Sample Agreement (95% CI)**	**Total Sample Agreement (95% CI)**
Site 1	Tech 1	97.5% (155/159) (93.7-99.0%)	80.3% (187/233) (74.7-84.9%)	87.2% (342/392) (83.6-90.2%)
	Tech 2	98.3% (174/177) (95.1-99.4%)	87.4% (188/215) (82.4-91.2%)	92.4% (362/392) (89.3-94.6%)
Site 2	Tech 1	100% (125/125) (97.0-100.0%)	85.3% (192/225) (80.1-89.4%)	90.6% (317/350) (87.1-93.2%)
	Tech 2	100% (139/139) (97.3-100.0%)	91.0% (192/211) (86.4-94.2%)	94.6% (331/350) (91.7-96.5%)
Site 3	Tech 1	100% (155/155) (97.6-100.0%)	87% (206/237) (82.0-90.7%)	92.1% (361/392) (88.9-94.4%)
	Tech 2	98.8% (167/169) (95.8-99.7%)	91.9% (205/223) (87.6-94.8%)	94.9% (372/392) (92.3-96.7%)

Combined Qualitative Agreement for Method A and Method C – When UNC from Method C Considered as Positive				
		Method A		**Total**
		Positive	**Negative**	
Method C	**Positive**	915	174	1089
	Negative	9	1170	1179
Total		924	1344	2268

PPA: 99.0 % (915/924) (95% CI: 98.4%; 99.6%)
NPA: 87.1 % (1170/1344) (95% CI: 85.2%; 88.7%)
Overall Agreement: 91.9% (2085/2268) (95% CI: 90.7%; 93.0%)

Qualitative Agreement between Method A and Method C (When UNC from Method C Considered as Negative)				
Method A vs Method C		**Positive Sample Agreement (95% CI)**	**Negative Sample Agreement (95% CI)**	**Total Sample Agreement (95% CI)**
Site 1	Tech 1	93.1% (148/159) (88.0-96.1%)	94.0% (219/233) (90.2-96.4%)	93.6% (367/392) (90.8-96.5%)
	Tech 2	90.4% (160/177) (85.2-93.9%)	99.1% (213/215) (96.7-99.7%)	95.2% (373/392) (92.6-96.9%)
Site 2	Tech 1	99.2% (124/125) (95.1-99.9%)	94.7% (213/225) (90.9-96.9%)	96.3% (337/350) (93.8-97.8%)
	Tech 2	95.0% (132/139) (89.9-97.5%)	98.1% (207/211) (95.2-99.3%)	96.9% (339/350) (94.5-98.2%)
Site 3	Tech 1	96.1% (149/155) (91.8-98.2%)	97.1% (230/237) (94.4-98.1%)	96.7% (379/392) (94.4-98.1%)
	Tech 2	90.5% (153/169) (85.2-94.1%)	98.7% (220/223) (96.1-99.5%)	95.2% (373/392) (92.6-96.9%)

Combined Qualitative Agreement for Method A and Method C – (When UNC from Method C Considered as Negative)				
		Method A		**Total**
		Positive	**Negative**	
Method C	**Positive**	866	42	908
	Negative	58	1302	1360
Total		924	1344	2268
PPA : 93.7 % (866/924) (95% CI: 92.0%; 95.1%) NPA: 96.9% (1302/1344) (95% CI: 95.8%; 97.7%) Overall Agreement: 95.6% (2168/2268) (95% CI: 94.7%; 96.4%)				

iii. Method B vs Method C Qualitative Comparison

Similar to the approach as presented in tables 10 through 13 above, UNC calls in Method C were counted towards both negatives as well as positives to determine the qualitative agreement between Method B versus Method C. The positive agreement, negative agreement and the total agreement between Method B versus Method C at each site and each technician as well combined for all sites and all technicians was similar to the results observed for Method A versus Method C.

b. **Pattern Agreement**

Additionally, pattern determinations were correlated across different interpretation methods for each sample included in the study. For the purpose of pattern recognition, samples that had no pattern (i.e., 'negative') or samples that yielded uncertain results (i.e., 'UNC') were included in the pattern agreement analyses between the different interpretation methods and are presented in the table below. Low titer samples, samples containing mixed patterns, and/or samples containing esoteric patterns (e.g., Golgi-like and centromere protein-F (CENP)-like patterns), were most likely to yield discrepant pattern results across interpretation methods and testing sites.

Pattern Agreement Between Methods at each Site and each Technician				
		Method A vs Method B	**Method A vs Method C**	**Method B vs Method C**
		% Agreement (95% CI)	**% Agreement (95% CI)**	**% Agreement (95% CI)**
Site 1	Tech 1	98.5% (386/392) (96.7-99.3%)	84.2% (330/392) (80.2-87.5%)	83.9% (329/392) (80.0-87.2%)
	Tech 2	97.7% (383/392) (95.7-98.8%)	85.0% (333/392) (81.1-88.2%)	85.2% (334/392) (81.4-88.4%)

Pattern Agreement Between Methods at each Site and each Technician				
		Method A vs Method B	**Method A vs Method C**	**Method B vs Method C**
		% Agreement (95% CI)	**% Agreement (95% CI)**	**% Agreement (95% CI)**
Site 2	Tech 1	96.9% (339/350) (94.5-98.2%)	86.0% (301/350) (82.0-89.3%)	86.9% (304/350) (82.9-90.0%)
	Tech 2	97.7% (342/350) (95.6-98.8%)	86.6% (303/350) (82.6-89.8%)	87.1% (305/350) (83.2-90.3%)
Site 3	Tech 1	98.47% (386/392) (96.7-99.3%)	88.3% (346/392) (84.7-91.1%)	88.5% (347/392) (85.0-91.3%)
	Tech 2	98.2% (385/392) (96.4-99.1%)	88.0% (345/392) (84.4-90.9%)	87.5% (343/392) (83.9-90.4%)

Combined Pattern Agreement for all Sites and all Technicians			
	Agreement #/ Total Samples	**% Agreement**	**95% CI**
Method A vs Method B	2221/2268	98.0%	97.3-98.4%
Method A vs Method C	1958/2268	86.3%	84.9-87.7%
Method B vs Method C	1962/2268	86.5%	85.0-87.9%

c. **End-point Titer Agreement**

Samples that were positive at each site were titrated to endpoint and interpreted by all three methods. Considering there were two technicians at each site to read by both Method A and Method B, this resulted in 886 comparisons in endpoint determinations between Method A versus Method B, Method A versus Method C and Method B versus Method C.

Combined Endpoint Titer Agreement between Methods for all Technicians and all Sites		
Interpretation	**+/- one dilution/Total samples**	**%Agreement (95% CI)**
Method A vs Method B	880/886	99.3% (98.5 - 99.7%)
Method A vs Method C	879/886	99.2% (98.4 - 99.6%)
Method B vs Method C	880/886	99.3% (98.5 - 99.7%)

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 190 samples associated with ANA-associated autoimmune diseases were tested, including systemic autoimmune rheumatic diseases (SARDs). At Site 1 and Site 3, 190 samples from diseases that usually are not associated with SARDs were tested; a smaller set of 160 samples was tested at Site 2. The samples were assayed at the screening 1:40 sample dilution then read by all three methods. At all three sites, Method A and Method B were interpreted by two different laboratory technicians. Samples positive at the 1:40 screening dilution were subsequently titered to determine the endpoint and pattern.

Clinically Characterized Samples used in the Study				
ANA-associated diseases	***n*, All Sites**	**Non- ANA-associated diseases**	***n*, Site 1 and Site 3**	***n*, Site 2**
SARDs:		*Other autoimmune diseases:*		
Systemic Lupus Erythematosus (SLE)	40	Celiac Disease	22	10
Sjögren's Syndrome (SS)	30	ANCA-associated Vasculitis	28	10
Scleroderma	20	Crohn's Disease	10	10
Autoimmune Myositis (AM)	20	Rheumatoid Arthritis	30	30
Mixed Connective Tissue Disease (MCTD)	20	Inflammatory Bowel Disease (IBD)	10	10
CREST	20	Autoimmune Thyroiditis	30	30
Other ANA-associated autoimmune diseases:		Ulcerative Colitis	10	10
Autoimmune Hepatitis (AIH)	20	*Other diseases:*		
Drug-induced Lupus (DIL)	20	Malignancy/cancer	20	20
		Fibromyalgia	10	10
		Infectious diseases	10	10
Total	190	**Total**	190	160

The clinical sensitivity was calculated at each site on SLE separately, and on the SARDs plus other ANA-associated diseases (autoimmune liver hepatitis + Drug-induced Lupus). Specificity was calculated using the non-ANA-associated diseases described above.

The clinical sensitivity and specificity for each site and each technician is presented in the table below:

Clinical Performance at Site 1		% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
		SLE (n = 40)	CTD + ANA-associated diseases (n = 190)	Non- ANA-associated diseases (n = 160)
Method A	Tech 1	52.5% (37.5-67.1%)	53.2% (46.1-60.1%)	75.8% (69.2-81.3%)
	Tech 2	57.5% (42.2-71.5%)	60.0% (52.9-66.7%)	73.2% (66.4-79.0%)
Method B	Tech 1	55.0% (39.8-69.3%)	54.7% (47.6-61.6%)	75.8% (69.2-81.3%)
	Tech 2	52.5% (37.5-67.1%)	57.9% (50.8-64.7%)	74.2% (67.6-79.9%)
Method C	dIFine	55.0% (39.8-69.3%)	57.4% (50.3-64.2%)	67.4% (60.4-73.6%)

Clinical Performance at Site 2		% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
		SLE (n = 40)	CTD + ANA-associated diseases (n = 190)	Non- ANA-associated diseases (n = 160)
Method A	Tech 1	50.0% (35.2-64.8%)	51.6% (44.5-58.6%)	83.1% (76.6-88.1%)
	Tech 2	55.0% (39.8-69.3%)	56.3% (49.2-63.2%)	80.0% (73.1-85.5%)
Method B	Tech 1	52.5% (37.5-67.1%)	56.8% (49.7-63.7%)	81.3% (74.5-86.5%)
	Tech 2	55.0% (39.8-69.3%)	58.4% (51.3-65.2%)	80.6% (73.8-86.0%)
Method C	dIFine	52.5%(37.5-67.1%)	55.8% (48.7-62.7%)	74.4% (67.1-80.5%)

Clinical Performance at Site 3		% Sensitivity (95% CI)	% Sensitivity (95% CI)	% Specificity (95% CI)
		SLE (n = 40)	CTD + ANA-associated diseases (n = 190)	Non- ANA-associated diseases (n = 190)
Method A	Tech 1	47.5% (32.9-62.5%)	53.2% (46.1-60.1%)	76.8% (70.4-82.3%)
	Tech 2	55.0% (39.8-69.3%)	57.4% (50.3-64.2%)	74.2% (67.6-79.9%)
Method B	Tech 1	45.0% (30.7-60.2%)	52.6% (45.6-59.6%)	78.9% (72.6-84.1%)
	Tech 2	57.5% (42.2-71.5%)	56.3% (49.2-63.2%)	74.7% (68.1-80.4%)
Method C	dIFine	55.0% (39.8-69.3%)	54.2% (47.1-61.1%)	67.5% (63.1-76.1%)

2. Percent Positivity

Percent positivity rates in the various disease cohorts by three different interpretation methods at the three sites are listed below:

Percent Positivity for all Disease Types at **Site 1**					
Diseases	**Technician 1**		**Technician 2**		
	Method A	**Method B**	**Method A**	**Method B**	**Method C**
ANA-associated Diseases					
SLE	52.5%	55.0%	57.5%	52.5%	55.0%
Sjögren's Syndrome	50.0%	50.0%	56.7%	53.3%	53.3%
Scleroderma	75.0%	70.0%	80.0%	80.0%	80.0%
Autoimmune Myositis	50.0%	55.0%	65.0%	60.0%	60.0%
MCTD	40.0%	40.0%	50.0%	55.0%	50.0%
CREST	80.0%	80.0%	80.0%	80.0%	80.0%
Autoimmune Hepatitis	45.0%	50.0%	60.0%	55.0%	50.0%
Drug-Induces Lupus	35.0%	40.0%	35.0%	35.0%	35.0%
Non-ANA associated Diseases					
Autoimmune Thyroiditis	23.3%	16.7%	16.7%	16.7%	16.7%
Cancer	15.0%	15.0%	20.0%	20.0%	15.0%
Celiac Disease	45.5%	45.5%	45.5%	45.5%	45.5%
Crohn's Disease	30.0%	30.0%	30.0%	30.0%	30.0%
Fibromyalgia	30.0%	40.0%	50.0%	40.0%	40.0%
Infectious Disease	5.0%	5.0%	5.0%	5.0%	5.0%
Inflammatory Bowel Disease	40.0%	30.0%	40.0%	20.0%	20.0%
Rheumatoid Arthritis	16.7%	23.3%	23.3%	23.3%	16.7%
Ulcerative Colitis	30.0%	30.0%	50.0%	60.0%	40.0%
Vasculitis	17.9%	14.3%	14.3%	14.3%	14.3%

Percent Positivity for all Disease Types at **Site 2**					
Diseases	**Technician 1**		**Technician 2**		
	Method A	**Method B**	**Method A**	**Method B**	**Method C**
ANA-associated Diseases					
SLE	50.0%	52.5%	55.0%	55.0%	52.5%
Sjögren's Syndrome	53.3%	53.3%	56.7%	56.7%	53.3%
Scleroderma	75.0%	80.0%	75.0%	80.0%	80.0%
Autoimmune Myositis	55.0%	65.0%	65.0%	70.0%	60.0%
MCTD	30.0%	45.0%	35.0%	45.0%	45.0%
CREST	75.0%	80.0%	80.0%	80.0%	75.0%
Autoimmune Hepatitis	40.0%	45.0%	45.0%	45.0%	45.0%
Drug-Induces Lupus	35.0%	40.0%	40.0%	40.0%	40.0%

Percent Positivity for all Disease Types at **Site 2**					
Diseases	**Technician 1**		**Technician 2**		
	Method A	**Method B**	**Method A**	**Method B**	**Method C**
Non-ANA associated Diseases					
Autoimmune Thyroiditis	13.3%	16.7%	16.7%	16.7%	16.7%
Cancer	10.0%	15.0%	15.0%	15.0%	15.0%
Celiac Disease	20.0%	30.0%	20.0%	30.0%	30.0%
Crohn's Disease	30.0%	30.0%	30.0%	30.0%	30.0%
Fibromyalgia	40.0%	40.0%	40.0%	40.0%	40.0%
Infectious Disease	5.0%	5.0%	5.0%	5.0%	5.0%
Inflammatory Bowel Disease	20.0%	20.0%	20.0%	20.0%	20.0%
Rheumatoid Arthritis	16.7%	16.7%	16.7%	16.7%	16.7%
Ulcerative Colitis	40.0%	40.0%	60.0%	40.0%	40.0%
Vasculitis	0.0%	0.0%	10.0%	10.0%	0.0%

Percent Positivity for all Disease Types at **Site 3**					
Diseases	**Technician A**		**Technician B**		
	Method A	**Method B**	**Method A**	**Method B**	**Method C**
ANA-associated Diseases					
SLE	47.5%	45.0%	55.0%	57.5%	52.5%
Sjögren's Syndrome	46.7%	50.0%	60.0%	53.3%	50.0%
Scleroderma	75.0%	75.0%	80.0%	75.0%	75.0%
Autoimmune Myositis	60.0%	60.0%	60.0%	60.0%	60.0%
MCTD	45.0%	45.0%	45.0%	45.0%	45.0%
CREST	80.0%	80.0%	75.0%	75.0%	75.0%
Autoimmune Hepatitis	45.0%	40.0%	50.0%	50.0%	45.0%
Drug-Induces Lupus	35.0%	35.0%	35.0%	35.0%	35.0%
Non-ANA associated Diseases					
Autoimmune Thyroiditis	16.7%	13.3%	20.0%	16.7%	16.7%
Cancer	20.0%	20.0%	20.0%	25.0%	15.0%
Celiac Disease	45.5%	45.5%	45.5%	45.5%	45.5%
Crohn's Disease	30.0%	20.0%	30.0%	30.0%	30.0%
Fibromyalgia	40.0%	40.0%	50.0%	50.0%	40.0%
Infectious Disease	5.0%	5.0%	5.0%	5.0%	5.0%
Inflammatory Bowel Disease	30.0%	30.0%	30.0%	30.0%	20.0%

Percent Positivity for all Disease Types at **Site 3**					
Diseases	**Technician A**		**Technician B**		
	Method A	**Method B**	**Method A**	**Method B**	**Method C**
Rheumatoid Arthritis	23.3%	16.7%	26.7%	20.0%	16.7%
Ulcerative Colitis	40.0%	40.0%	60.0%	60.0%	40.0%
Vasculitis	10.7%	10.7%	10.7%	14.3%	14.3%

3. Other Clinical Supportive Data (When 1. and 2. Are Not Applicable):

Not applicable

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 assayed at the 1:40 screening titer using the ZEUS IFA ANA HEp-2 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. Percent positivity was determined for the 1:40 screening data. The results for the reference range are summarized below in the following table:

Reference Range Determination (N=180)						
Method	**Number of Positives**	**% Positives**	**Number of Negatives**	**% Negatives**	**Number of Uncertain**	**% Uncertain**
A	19	10.6%	161	89.4%	NA	NA
B	19	10.6%	161	89.4%	NA	NA
C	14	7.8%	152	84.4%	14	7.8%

F Other Supportive Instrument Performance Characteristics Data:

Not Applicable

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.