

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM
ASSAY AND INSTRUMENT COMBINATION TEMPLATE**

A. 510(k) Number:

K160265

B. Purpose for Submission:

New instrument for a cleared IVD assay

C. Measurand:

Anti-nuclear antibody (ANA)

D. Type of Test:

Qualitative and/or semi-quantitative, indirect immunofluorescence

E. Applicant:

Immuno Concepts, NA, Ltd.

F. Proprietary and Established Names:

HEp-2000®Fluorescent ANA/Ro Test System
Image Navigator by Immuno Concepts

G. Regulatory Information:

1. Regulation section:

21 CFR 866.5100 – Antinuclear antibody immunological test system

2. Classification:

Class II

3. Product code:

DHN – Antinuclear Antibody, Indirect Immunofluorescent, Antigen, Control
PIV – Automated indirect immunofluorescent microscope and software-assisted system
for clinical use

4. Panel:

Immunology (82)

H. Intended Use:

1. Intended use(s):

Instrument:

The Immuno Concepts Image Navigator is an automated system consisting of a fluorescent microscope and software that acquires, interprets, stores, and displays digital images of stained indirect immunofluorescent slides. The Image Navigator can only be used with cleared or approved Immuno Concepts in vitro diagnostic assays that are indicated for use on the microscope. All suggested positive/negative results generated by the Image Navigator software must be confirmed by trained laboratory personnel.

Assay:

This is an indirect fluorescent antibody test for the semi-quantitative detection of IgG antinuclear antibody (ANA) in human serum by manual fluorescence microscopy or with the Image Navigator Fluorescence Semi-Automated Microscope. This test system uses transfected HEP-2000 cells, which allow specific identification of autoantibodies to the SSA/Ro antigen. Antibodies to SSA/Ro may show a distinctive staining pattern on the transfected cells. When this pattern is present, it is considered to be confirmatory evidence that anti-SSA/Ro antibodies are present.

Absence of this distinctive pattern does not rule out the possible presence of anti-SSA/Ro antibodies

This test system is to be used as an aid in the detection of antibodies associated with systemic rheumatic disease in conjunction with other laboratory and clinical findings. A trained operator must confirm results generated with the Image Navigator semi-automated device and software.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

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

4. Special instrument requirements:

Immuno Concepts Image Navigator is an automated system consisting of a fluorescent

microscope and software that provides results as possibly negative and possibly positive. A trained operator must confirm the results.

I. Device Description:

Assay kit components:

- Substrate Slides: ANA substrate slides using HEp-2000 cells (with mitotic figures), 7-well, 14-well, 18-well, and 21-well
- SSA/Ro Positive Control, ready-to-use
- Homogeneous Positive Control, ready-to-use
- Speckled Positive Control, ready-to-use
- Nucleolar Positive Control, ready-to-
- Centromere Positive Control, ready-to-use
- Titratable Control Serum, ready-to-use
- Negative Control Serum, ready-to-use, it demonstrates no discernible pattern of nuclear staining.
- Fluorescent Antibody Reagent, Goat anti-human IgG (heavy and light chain) conjugated to fluorescein isothiocyanate (FITC), ready-to-use
- PBS Buffer Powder
- Mounting Medium
- Coverslips

Materials required but not provided:

- Fluorescent microscope equipped with 495 nm exciter filter and 515 nm barrier filter

J. Substantial Equivalence Information:

1. Predicate device name(s):

HEp-2000 Fluorescent ANA/Ro Test System from Immuno Concepts, NA, Ltd

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

K972145

3. Comparison with predicate:

Similarities		
Item	Device HEp-2000® Fluorescent ANA/Ro Test System	Predicate HEp-2000® Fluorescent ANA/Ro Test System
Intended Use	Indirect fluorescent antibody test for the semi-quantitative detection of IgG antinuclear antibody (ANA) in human serum by manual fluorescence	Same

Similarities		
Item	Device HEp-2000® Fluorescent ANA/Ro Test System	Predicate HEp-2000® Fluorescent ANA/Ro Test System
	microscopy or with the Image Navigator Fluorescence Semi-Automated Microscope. This test system uses transfected HEp-2000® cells, which allow specific identification of autoantibodies to the SSA/Ro antigen. Antibodies to SSA/Ro may show a distinctive staining pattern on the transfected cells. When this pattern is present, it is considered to be confirmatory evidence that anti-SSA/Ro antibodies are present	
Method	Indirect Immunofluorescence (IFF)	Same
Reported result	Qualitative, semi-quantitative	Same
Sample matrix	Serum	Same
Analyte	ANA of IgG isotype	Same
Antigen	HEp-2000	Same
Slides	7-well, 14-well, 18-well and 21 well coated with antigen	Same
Conjugate	FITC conjugated goat anti-human IgG (heavy and light chain)	Same
Controls	Titrateable control, Negative control, Positive control for the following patterns: SSA/Ro, Homogeneous, Speckled, Nucleolar, Centromere	Same
Storage	2–10°C	Same
Sample dilution	1:40	Same

Differences		
Item	Device HEp-2000® Fluorescent ANA/Ro Test System	Predicate HEp-2000® Fluorescent ANA/Ro Test System
Instrument	The Image Navigator is an automated microscope designed to capture and display “possibly positive” and “possibly negative” images for the user	Conventional fluorescent microscope that the user employs to visualize the images.

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

Guidance for Industry and FDA Staff: Recommendations for Anti-Nuclear Antibody (ANA) Test System Premarket (510(k)) Submissions (January 22 2009)

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition, 5/21/07, CLSI

L. Test Principle:

The Immuno Concepts Fluorescent ANA Test System uses the indirect fluorescent antibody technique. The test system uses human epithelial cells (HEp-2 cells) that have been transfected with SSA/Ro autoantigen (HEp-2000 cell with mitotic figures). Patient samples are diluted 1:40 in PBS and are incubated with antigen substrate (HEp-2000 cells) on the slide to allow specific binding of autoantibodies to cell nuclei. If ANA antibodies are present, a stable antigen-antibody complex is formed. After washing to remove non-specifically bound antibodies, the substrate is incubated with an anti-human antibody conjugated to fluorescein. When results are positive, there is the formation of a stable three-part complex consisting of fluorescent antibody bound to human antinuclear antibody, which is bound to nuclear antigen. This complex can be visualized with the aid of a fluorescent microscope. In positive samples, the cell nuclei will show an apple-green fluorescence with a staining pattern characteristic of the particular nuclear antigen distribution within the cells. If the sample is negative for ANA, the nucleus will not show a clearly discernible pattern of nuclear fluorescence.

Stained slides can be read by conventional fluorescent microscopy, or scanned with the Image Navigator. The Image Navigator will separate the images into two broad categories, “Possibly Positive” and “Possibly Negative”. The trained users can then review the images on the computer monitor, confirm the positive/negative designation, and determine the pattern(s) of ANA staining. The kit includes positive controls for the five most common patterns.

Samples that are positive at the screening dilution may be titered manually using two-fold serial dilutions with PBS buffer solution to determine the endpoint titer.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

Nomenclature:

Modes for comparison:

Mode	Imaging	Reading
Conventional	Manual	Manual, on the microscope
Monitor	Automated, images captured and	Manual, reading

	displayed by the Image Navigator and are not separated into “possibly positive” and “possibly negative”	images on the computer monitor
Image Navigator	Automated, images separated and displayed by the Image Navigator into “possibly positive” and “possibly negative”	Manual, reading images on the computer monitor

The Image Navigator does not recognize patterns. The kit has positive controls for the following patterns:

Pattern	Abbreviation
SSA/Ro	SSA/Ro
Homogeneous	H
Speckled	S
Nucleolar	N
Centromere	C

The Image Navigator does not recognize fluorescence intensity. The following fluorescence intensity is provided as a guide for operators performing their own analyses:

Intensity	Interpretation
4+	Brilliant yellow-green (maximal fluorescence): clear-cut cell outline; sharply defined cell center.
3+	Less brilliant yellow-green fluorescence: clear-cut cell outline; sharply defined cell center.
2+	Definite cell pattern but dim fluorescence: cell outline less well defined.
1+	Very subdued fluorescence: cell outline almost indistinguishable from cell center in most instances.

The Image Navigator does not recognize or recommend titers. The following serves as a guidance for operators performing their own analyses:

Titer	Consideration
1:40 to 1:80	Considered low titers
1:160 to 1:320	Considered medium titers
1:640 and greater	Considered high titers

Interpretation of results:

Negative: A serum is considered negative for antinuclear antibodies if nuclear staining is less than or equal to the negative control well with no clearly discernible pattern. The cytoplasm may demonstrate weak staining, with brighter staining of the nonchromosome region of mitotic cells, but with no clearly discernible nuclear pattern.

Positive: A serum is considered positive if the nucleus shows a clearly discernible pattern of staining in a majority of the interphase cells.

SSA/Ro: A serum is considered positive for SSA/Ro antibodies if 10–20% of the

interphase nuclei show the distinctive SSA/Ro staining pattern, which appears as a distinct bright speckled pattern with prominent staining of the nucleoli. These are the over-expressing transfected cells. The remaining 80–90% of the interphase nuclei may or may not demonstrate a fine speckled staining of the nucleus with or without fluorescent staining of the nucleoli.

Titers: When reading titers, it is recommended to begin reading with the well that contains the most dilute sample and read “backwards” to the 1:40 dilution. The first well in which a clearly discernible pattern is visible is the titer end point. It is important that the intensity of staining not be confused with the presence or absence of antinuclear antibodies. The key factor to consider in determining whether a given dilution of serum is positive is the appearance of a clearly discernible nuclear pattern, irrespective of the staining intensity. Due to the increased concentration of SSA/Ro antigen in the over-expressing cells, it is not unusual to see staining of these cells in very high titers. The clinical significance of these high titers is unknown.

CAUTION: Some sera may demonstrate nuclear and cytoplasmic staining with no apparent nuclear pattern. This phenomenon is generally due to heterophile antibodies and should be reported as negative.

Reporting of results:

Screening: Results should be reported as possibly positive at the 1:40 dilution, and the nuclear staining pattern should be reported.

Titering: Samples can be manually diluted to evaluate titers. Results should be reported as the last serial dilution in which clearly discernible staining is seen. Results with a strong reaction at the 1:2560 dilution should be reported as greater than 1:2560. Titers of 1:40 to 1:80 are considered low titers; 1:160 to 1:320 are considered medium titers; and 1:640 and greater are considered high titers.

a. Precision/Reproducibility:

Repeatability:

To assess repeatability of the assay using Conventional Reading, Monitor Reading, and the classification as possibly positive or negative by the Image Navigator, ten positive and ten negative samples were run. The positive samples included commonly encountered patterns and covered staining intensities from 2+ to 4+. The negative samples included four samples that were considered “High Intensity Negative” when they were originally read on a conventional fluorescent microscope. The samples were tested in three replicates each in ten runs on ten consecutive working days (N = 30). Samples were randomized so that the order of appearance on the slides varied from one run to the next. The results of this study are shown in the table below. There were no discrepancies among the positive samples. Two of the negative samples showed discrepancies. The sponsor clarified that the discrepancies were distributed among the ten daily runs and did not show any pattern or trend. The

two samples that showed discrepancies were originally categorized as “High Intensity Negative” when they were originally read on a conventional fluorescent microscope in a clinical laboratory. These samples represent the “borderline” samples that are confusing and problematic for laboratory personnel, no matter what reading modality is used.”

Two Discrepant samples:

Samples	Conventional	Monitor	Image Navigator
Positive (N = 300)	No discrepancies	No discrepancies	No discrepancies
Negative (N = 300)	3/300	3/300	3/300

Reproducibility:

Between-operator and between-instrument variability was assessed by testing a cohort of 148 samples at the three testing sites. Three users at each site interpreted the images. Reproducibility was assessed for Within-Site, Between-Site, and Between Operator Agreement. The studies were done on three Image Navigator instruments, one at each site.

Within Site Agreement:

N = 148	Reading Method	Positive Agreement % (95% CI)	Negative Agreement % (95% CI)	Overall Agreement % (95% CI)
Site 1	Conventional vs. Monitor	69/70 = 98.6% (92.3–99.9)	77/78 = 98.7% (93.1–99.9)	146/148 = 98.7% (95.2–99.8)
	Monitor vs. Image Navigator	69/70 = 98.6% (92.3–99.9)	77/78 = 98.7% (93.1–99.9)	146/148 = 98.7% (95.2–99.8)
	Conventional vs. Image Navigator	68/70 = 97.1% (90.1–99.7)	78/78 = 100% (95.4–100.0)	146/148 = 98.7% (95.2–99.8)
Site 2	Conventional vs. Monitor	67/67 = 100% (94.6–100)	80/81 = 98.8% (93.3–99.8)	147/148 = 99.3% (96.3–99.9)
	Monitor vs. Image Navigator	66/68 = 97.1% (89.8–99.6)	79/80 = 98.8% (93.2–99.9)	145/148 = 98.0% (94.2–99.6)
	Conventional vs. Image Navigator	65/67 = 97.0% (89.6–99.6)	79/81 = 97.5% (91.4–99.7)	144/148 = 97.3% (93.2–99.3)
Site 3	Conventional vs. Monitor	65/68 = 95.6% (87.6–99.1)	76/80 = 95.0% (87.7–98.6)	141/148 = 95.3% (90.5–98.1)
	Monitor vs. Image Navigator	66/69 = 95.7% (87.8–99.1)	77/79 = 97.5% (91.2–99.7)	143/148 = 96.6% (92.3–98.9)
	Conventional vs. Image Navigator	66/68 = 97.1% (89.8–99.6)	78/80 = 97.5% (91.3–99.7)	144/148 = 97.3% (93.2–99.3)

Between site reproducibility, conventional reading:

Conventional Reading N = 148	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Positive Agreement % (95% CI)	65/70 = 92.9% (84.1–97.6)	65/70 = 92.9% (81.4–97.6)	65/67 = 92.9% (94.7–100.0)

Negative Agreement % (95% CI)	76/78 = 97.4% (91.0–99.7)	75/78 = 96.2% (89.2–99.2)	78/81 = 96.3% (89.6–99.2)
Overall Agreement % (95% CI)	141/148 = 95.3% (90.5–98.1)	140/148 = 94.6% (89.6–97.3)	143/148 = 96.6% (92.3–98.9)

Between site reproducibility, monitor reading:

Monitor Reading N = 148	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Positive Agreement % (95% CI)	66/70 = 94.3% (86.0–98.4)	67/70 = 95.7% (92.1–100.0)	66/68 = 97.1% (89.8–99.6)
Negative Agreement % (95% CI)	76/80 = 95.0% (87.7–98.6)	76/78 = 97.4% (91.0–99.7)	77/80 = 96.3% (89.4–99.2)
Overall Agreement % (95% CI)	142/148 = 95.6% (91.4–98.5)	143/148 = 96.6% (92.3–98.9)	143/148 = 96.6% (92.3–98.9)

Between site reproducibility, Image Navigator reading:

Image Navigator N = 148	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Positive Agreement % (95% CI)	65/68 = 95.6% (87.6–99.1)	65/68 = 95.6% (87.6–99.1)	66/67 = 98.5% (92.0–99.9)
Negative Agreement % (95% CI)	78/80 = 97.5% (91.3–99.7)	77/80 = 96.3% (89.4–99.2)	79/81 = 97.5% (91.4–99.7)
Overall Agreement % (95% CI)	143/148 = 96.6% (92.3–98.9)	142/148 = 95.6% (91.4–98.5)	145/148 = 98.0% (94.2–99.6)

Between Operator and between Instrument Agreement: the same cohort of 148 samples was tested at the three testing sites. The results are summarized in the table below:

N = 148		Positive Agreement % (95%CI)	Negative Agreement % (95%CI)	Overall Agreement % (95%CI)
Site 1	Conventional vs monitor	100 (94.7–100)	98.8 (93.2–100)	99.3 (96.3–100)
	Monitor vs Image Navigator	100 (94.7–100)	100 (95.5–100)	100 (97.5–100)
	Conventional vs Image Navigator	100 (94.7–100)	98.8 (93.2–100)	99.3 (96.3–100)
Site 2	Conventional vs monitor	97.1 (89.8–99.6)	98.8 (96.6–99.9)	98.0 (94.2–99.8)
	Monitor vs Image Navigator	98.5 (92.1–100)	98.8 (96.6–99.9)	98.6 (95.2–99.8)
	Conventional vs Image Navigator	98.5 (92.1–100)	100 (95.5–100)	99.3 (96.3–100)
	Conventional vs monitor	97.1 (92.1–100)	98.8 (96.6–99.9)	98.0 (94.2–99.8)

Site 3	Monitor vs Image Navigator	98.5 (92.1–100)	97.5 (91.3–99.7)	98.0 (94.2–99.8)
	Conventional vs Image Navigator	100 (97.4–100)	98.8 (93.2–100)	99.3 (96.3–100)

Fluorescence intensity:

The image Navigator does not assign intensity grades as part of the assessment of the positivity/negativity of a sample, so any estimate of intensity would require the user to look at the image on the monitor, and would therefore be the same as the assessment from the monitor. However, given that the user may evaluate intensity on the monitor, a comparison between conventional and monitor was performed. The fluorescent intensity grades were within \pm one grade from each other between conventional reading and monitor reading, as shown in this table:

N = 148	Percent of samples within \pm one grade		
	Site 1	Site 2	Site 3
Conventional Vs Monitor	147/148 99.30%	146/148 98.60%	147/148 99.30%

Lot-to-Lot Comparison: The reagents for this assay were cleared in K972145. A new lot-to-lot reproducibility study was performed using three reagent lots and the data showed > 90% positive/negative and total agreement between the three lots.

b. *Linearity/assay reportable range:*

Not applicable:

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

Traceability: A recognized standard for Anti-Nuclear Antibodies is not available.

Controls:

The Immuno Concepts HEp-2000 kit includes the five major patterns positive controls (Homogeneous, Speckled, Nucleolar, Centromere, and SSA/Ro) as well as a negative control and titratable control serum. The sponsor recommends running positive, negative and PBS controls on each slide. For general screening, the homogeneous positive control is recommended.

Stability: The kit used with the Image Navigator is the same kit that has been in commercial distribution since 1997, stability has been previously established. All components of the HEp-2000 Fluorescent ANA kit are stable for at least 12 months from the date of manufacture.

d. *Detection limit:*

Not applicable

e. *Analytical specificity:*

Interference:

Interference was assessed using the low, medium and high testing concentrations. No interference was detected up to the concentrations listed in the table below:

Interfering Substance	No Interference up to concentrations below:
Bilirubin, conjugated	10 mg/dL
Hemoglobin	200 mg/dL
Triglycerides	1000 mg/dL
Cholesterol	230 mg/dL
IgM RF	60 units

Cross Reactivity:

The CDC ANA reference standards (also known as IUIS ANA reference standards) were tested with the HEp-2000 fluorescent ANA kit, and scanned with the Image Navigator microscope. The trained user interpreted and confirmed the images that were presented on the monitor. Additionally, the same operator read the slides with a conventional fluorescent microscope.

All reference sera showed the same results (as possibly positive and possibly negative) when compared to the conventional reading and the monitor reading. There were no discrepancies in pattern interpretation between conventional and instrument results. The results of interpretation from the computer monitor were within ± 1 fluorescence intensity grade from that of conventional interpretation of the slides.

In Human Reference Serum #7, which has known antibody specificity to the SSA/Ro antigen, the distinctive SSA/Ro pattern was detectable by both conventional reading and monitor reading.

f. *Assay cut-off:*

The cut off is the same as the predicate K972145. For the HEp-2000 Fluorescent ANA test kit, the cut-off was previously established as a 1:40 dilution. For immunofluorescent tests, the starting dilution establishes the cut-off for the assay. In the ANA assay, a clearly discernable pattern of nuclear or cytoplasmic staining at the screening dilution is considered positive, and the absence of a clear pattern is considered negative.

2. Comparison studies:

a. *Method comparison with predicate device:*

The Conventional assessment serves as the predicate as the device already was cleared in K972145. Clinical samples (n=739) at each site were read on one instrument and by three readers. Agreement between the three reading methods and between the three sites was evaluated. The results are summarized in the tables below:

Within site agreement between methods:

N = 739	Overall Agreement % (95% CI)		
	Site 1	Site 2	Site 3
Conventional vs. Monitor	99.3% (98.4–99.8)	99.6% (98.8–99.9)	99.6% (98.8–99.9)
Image Navigator vs. Monitor	99.7% (99.0–99.9)	100% (99.5–100)	100% (99.5–100)
Image Navigator vs. Conventional	99.3% (98.4–99.8)	99.3% (98.4–99.8)	99.6% (98.8–99.9)

Between site agreement between methods:

N = 739	Overall Agreement % (95% CI)		
	Site 1 vs. Site 2	Site 1 vs. Site 3	Site 2 vs. Site 3
Conventional Reading	732/739 = 99.1% (98.0–99.6)	732/739 = 94.6% (98.0–99.6)	730/739 = 98.8% (97.7–99.4)
Monitor	734/739 = 99.3% (98.4–99.8)	736/739 = 99.6% (98.8–99.9)	734/739 = 99.3% (92.3–98.9)
Image Navigator	734/739 = 99.3% (98.4–99.8)	738/739 = 99.9% (99.3–99.9)	733/739 = 99.2% (98.2–99.7)

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

To assess the clinical sensitivity and specificity and accuracy of the Image Navigator, the same cohort of 739 clinically characterized samples were evaluated using the Image Navigator. Images were separated into “Possibly Positive” and “Possibly Negative” by the Image Navigator software and confirmed by three independent users. The images were also read on the monitor by the same three users without interpretation by the software, and the same slides were read by the same three users using conventional fluorescent microscopy. The number of samples and corresponding diagnoses are shown the following table:

Autoimmune Diseases Often Associated with Antinuclear Antibodies	N
Systemic Lupus Erythematosus	97
Cutaneous Subacute Lupus Erythematosus	25
Acute Cutaneous Lupus Erythematosus	12
Neonatal Lupus Erythematosus	3

Drug-Induced Lupus Erythematosus	10
Sjögren's Syndrome	53
Scleroderma	28
Mixed Connective Tissue Disease	28
Autoimmune Myositis*	23
Autoimmune Liver Disease*	22
Total	301
Autoimmune Diseases in Which Antinuclear Antibodies Are Not Part of the Diagnostic Criteria	
Autoimmune Vasculitis	32
Antiphospholipid Syndrome	16
Rheumatoid Arthritis	25
Fibromyalgia	9
Total	82
Diseases that are Generally Not Associated with Antinuclear Antibodies	
Gluten-sensitive Enteropathy	18
Inflammatory Bowel Disease	16
Pernicious Anemia	12
Multiple Myeloma	4
Nonspecific Joint Pain	52
Autoimmune Thyroiditis	26
Total	128
Infectious Diseases	
EBV	26
CVM	27
HBV	8
HCV	6
HIV	3
Syphilis	16
Total	86
Healthy Control	
Total	142
Total Samples	739

Summary of Patterns in study:

Pattern Abreviation	Pattern Name	Number of samples
Neg	Negative	421
H	Homogeneous	43
S	Speckled	104
N	Nucleolar	7
C	Centromere	5
SSA (sometimes SSA/Ro)	SSA	31
H/S (or S/H)	Mixes Homogeneous and	49

	Speckled	
H/SSA	Mixes Homogeneous and SSA	7
S/SSA	Mixes Speckled and SSA	35
H/S/SSA	Mixes Homogeneous Speckled and SSA	2
H/N (or N/H)	Mixes Homogeneous and Nucleolar	3
S/N (or N/S)	Mixes Speckled and Nucleolar	3
H/S/N	Mixes Homogeneous Speckled and Nucleolar	13
AMA (Cytoplasmic)	Anti-Mitochondrial Antibody	13
ASMA (cytoplasmic)	Anti-Smooth Muscle Antibody	6
	Total	739

a. *Clinical Sensitivity and specificity :*

Percent positive samples, average of three sites and three readers per site

Sample Type	N	Conventional Microscopy	Monitor Reading	Image Navigator
Autoimmune Diseases Often Associated with Antinuclear Antibodies (N = 301)				
SLE	97	94.0%	94.2%	94.1%
SCLE	25	73.3%	75.6%	76.4%
ACLE	12	71.3%	71.3%	73.1%
NLE	3	100.0%	100.0%	100.0%
DIL	10	61.1%	61.1%	61.1%
Sjögren's Syndrome	53	87.4%	87.8%	87.8%
Scleroderma	28	86.1%	85.7%	85.7%
MCTD	28	94.4%	95.6%	94.8%
Autoimmune Myositis	23	30.4%	29.5%	29.0%
Autoimmune Liver Disease	22	80.8%	81.3%	81.1%
Autoimmune Diseases in Which Antinuclear Antibodies Are Not Part of the Diagnostic Criteria (N = 82)				
Autoimmune Vasculitis	32	20.5%	21.2%	21.5%
Antiphospholipid Syndrome	16	52.1%	51.4%	52.1%
Rheumatoid Arthritis	25	40.4%	40.0%	40.0%
Fibromyalgia	9	11.1%	11.1%	11.1%
Diseases that are Generally Not Associated with Antinuclear Antibodies (N = 128)				
Gluten-Sensitive Enteropathy	18	11.1%	11.1%	11.1%

Inflammatory Bowel Disease	16	6.3%	6.3%	6.3%
Pernicious Anemia	12	0.0%	0.0%	0.0%
Multiple Myeloma	4	0.0%	0.0%	0.0%
Nonspecific Joint Pain	52	13.5%	13.5%	13.5%
Autoimmune Thyroiditis	26	19.2%	19.2%	19.2%
Infectious Diseases (N = 86)				
Acute EBV Infection	26	7.7%	7.7%	7.7%
Acute CMV Infection	27	11.1%	11.1%	11.1%
Hepatitis B Infection	8	0.0%	0.0%	0.0%
Hepatitis C Infection	6	0.0%	0.0%	0.0%
HIV Infection	3	0.0%	0.0%	0.0%
Syphilis (Primary or Secondary)	16	0.0%	0.0%	0.0%
Healthy Controls (N = 142)				
Healthy Control	142	6.9%	6.4%	6.0%
Total	739			

Sensitivity, that is, a result of “possibly positive” for true positive samples, was calculated for the various disease states. Because of small numbers of samples for some disease states, sensitivity was assessed for SLE alone, SLE combined with other lupus-associated diseases (SCLE, ACLE, and NLE), and the combination of connective tissue diseases (CTD) (SLE + Sjögren’s Syndrome + Scleroderma + MCTD + autoimmune myositis + DIL + autoimmune liver disease (AIL). Specificity, that is, a result of “possibly negative” for true negative samples, was calculated on the control population (autoimmune diseases in which antinuclear antibodies are not part of the diagnostic criteria (n = 82), diseases that are generally not associated with antinuclear antibodies (n=128), and infectious diseases (n = 86)) excluding healthy controls, and separately including the healthy controls. The results are summarized in the tables below:

Site 1	Sensitivity % (95% CI)			Specificity % (95% CI)	
	SLE alone N = 97	All Lupus N = 137	CTD + AIL N = 301	Excluding Healthy N = 296	Including Healthy N = 438
N = 739					
Conventional	94.8 (88.4–98.9)	89.1 (85.3–93.7)	82.4 (78.0–86.8)	84.5 (79.8–88.4)	87.2 (83.7–90.2)
Monitor	94.8 (88.4–98.3)	89.8 (83.5–94.3)	83.4 (78.7–87.4)	84.5 (79.8–88.4)	87.2 (83.7–90.2)
Image Navigator	94.8 (89.8–98.9)	89.1 (85.3–93.7)	83.1 (78.3–87.1)	84.1 (79.5–88.1)	87.9 (84.5–90.8)

Site 2	Sensitivity % (95% CI)			Specificity % (95% CI)	
	SLE alone N = 97	All Lupus N = 137	CTD + AIL N = 301	Excluding Healthy N = 296	Including Healthy N = 438
N = 739					

Conventional	93.8 (87.0–97.7)	89.1 (85.3–93.7)	82.7 (78.0–86.8)	84.5 (79.8–88.4)	87.4 (84.0–90.4)
Monitor	95.9 (88.4–98.3)	89.1 (85.3–93.7)	82.7 (78.0–86.8)	84.5 (79.8–88.4)	87.4 (84.0–90.4)
Image Navigator	94.8 (88.4–98.3)	89.8 (83.5–94.3)	83.4 (78.7–87.4)	84.5 (79.8–88.4)	87.2 (83.7–90.2)

Site 3	Sensitivity % (95% CI)			Specificity % (95% CI)	
N = 739	SLE alone N = 97	All Lupus N = 137	CTD+AIL N = 301	Excluding Healthy N = 296	Including Healthy N = 433
Conventional	92.8 (85.7–97.1)	87.6 (80.9–92.6)	82.7 (78.0–86.8)	84.5 (79.8–88.4)	87.2 (83.7–90.2)
Monitor	92.8 (85.7–97.1)	88.3 (81.7–93.2)	82.7 (78.0–86.8)	84.5 (79.8–88.4)	87.4 (84.0–90.4)
Image Navigator	92.8 (85.7–97.1)	89.1 (85.3–93.7)	82.7 (78.0–86.8)	84.5 (79.8–88.4)	87.2 (83.7–90.2)

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

Not applicable

4. Clinical cut-off:

See assay cut-off

5. Expected values/Reference range:

The following data was taken from the predicate K972145. In a large university medical center, using HEP-2000 cell ANA substrate, data from over 4500 individual patients were generated over a two-year period:

Diagnosis	Pattern	% Positive
Abnormal Population:		
Systemic lupus erythematosus	S, H, H/S	93
Rheumatoid arthritis	S, H	40
Mixed connective tissue disease	S	99
Progressive systemic sclerosis–diffuse	S, N	85
Progressive systemic sclerosis–CREST	C	93
Sjögren’s Syndrome	S, SSA	92
Autoimmune myositis	S, Cyto	25
Autoimmune Vasculitis	S	20

Apparently Healthy Population (Over 9,000 Sera Tested):		
20–60 Years	S, H/S	2
61–80 Years	S, H/S	3.5

Abbreviations: S = Speckled, H = Homogeneous, H/S = Mixed Homogeneous and Speckled, N = Nucleolar, C = Centromere, Cyto = Cytoplasmic

The reference range for detection of antinuclear antibodies in the normal population is “Negative”. As shown in the Table above, a small percentage of normal individuals will exhibit a positive ANA. Each laboratory should establish and maintain its own reference (normal) range values, based on the patient population and other local factors.

N. Instrument Name:

Image Navigator

O. System Descriptions:

Image Navigator is an automated scanning microscope and image analysis system. The system is comprised of a computer, monitor, keyboard, mouse, barcode readers, installed software, microscope, and digital camera. The automated microscope includes a motorized stage, autofocus drive, bright field lamp, LED fluorescence lamp, eyepieces, condenser, and camera adapter. Images captured by the digital camera are transferred to the computer for entry into a file appropriate to the patient identification, and subsequent analysis by the software algorithm. All of the hardware components are standard “off-the-shelf” items.

The immunofluorescent slides used for antinuclear antibody testing are processed and stained in the usual manner, following the recommendations of the manufacturer of the antinuclear antibody testing kit. A unique barcode label identifies each slide. The software associated with the system associates this barcode label with the identification of the patients and controls that are in the various wells on the antinuclear antibody slide.

After the immunofluorescent slide has been prepared, the user scans the barcode label and places the slide in one of the four positions on the microscope stage, as directed by the software. The user opens the image acquisition program, selects the slide format (7, 14, 18, or 21 well) and the software begins the run. The software moves the motorized stage and reads the barcode on the slide. The software confirms that the slide is in the correct position on the stage and determines the samples that are on the slide. Slide format selection indicates to the software the X, Y coordinates of each of the wells on the slide. The system then performs an autofocus check, moves to the first well on the first slide, focuses on the cells, and captures four digital images. These four images are stored in the appropriate file on the computer. The motorized stage moves automatically to the next well, focuses, and captures four images. This process continues until all images of all wells on all slides have been captured. This process does not require any user intervention.

After all of the slides in the batch have been processed, the user initiates analysis of the run.

In the first step of analysis, the software separates the samples into two groups: those that are *possibly negative* for anti-nuclear antibodies, and those that are *possibly positive* for anti-nuclear antibodies. The user will review the slides and pick the pattern. Additional titration can be performed manually and slides read again on the Image Navigator.

1. Modes of Operation:

Manual mode (manual imaging with manual reading) and semi-automated modes (automated imaging with manual reading and/or positive/negative suggested results only)

Does the applicant's device contain the ability to transmit data to a computer, webserver, or mobile device?

Yes or No

Does the applicant's device transmit data to a computer, webserver, or mobile device using wireless transmission?

Yes or No

2. Software:

Software Version – 1.5.001 dated 8/05/2013

Level of Concern: Moderate

FDA has reviewed applicant's Hazard Analysis and software development processes for this line of product types:

Yes or No

3. Specimen Identification:

Manual sample identification and/or Barcode

4. Specimen Sampling and Handling:

Not applicable

5. Calibration:

A Calibration Verification for the Image Navigator system is to be run monthly and is performed by using a red Calibration Slide, which is supplied by Immuno Concepts. The procedure is included in the User Manual.

6. Quality Control:

Titrateable control, negative control, and positive controls are supplied with the assay reagents (see assay description above)

P. Other Supportive Instrument Performance Characteristics Data Not Covered In The “Performance Characteristics” Section above:

N/A

Q. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

R. Conclusion:

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