

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION MEMORANDUM**

**A. 510(k) Number:**

k150155

**B. Purpose for Submission:**

New assay

**C. Measurand:**

Anti-Nuclear Antibodies (ANA)

**D. Type of Test:**

Qualitative and/or semi-quantitative, indirect immunofluorescence

**E. Applicant:**

Inova Diagnostics, Inc.

**F. Proprietary and Established Names:**

NOVA Lite® DAPI ANA Kit

**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 immunofluorescence microscope and software-assisted system for clinical use

4. Panel:

Immunology (82)

**H. Intended Use:**

1. Intended use(s):

NOVA Lite® DAPI ANA Kit is an indirect immunofluorescence assay for the qualitative detection and semi-quantitative determination of anti-nuclear antibodies of the IgG isotype in human serum by manual fluorescence microscopy or with the NOVA View Automated Fluorescence Microscope. 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. A trained operator must confirm results when generated with the NOVA View device.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only.

All software-aided results must be confirmed by the trained operator.

4. Special instrument requirements:

NOVA View® Automated Fluorescence Microscope using NOVA View® Software Version 2.1.3 (DEN140039) or manual fluorescence microscope equipped with a mercury vapor or LED light source, a 460-495 nm excitation filter and a 510-520 nm barrier filter.

**I. Device Description:**

The NOVA Lite® DAPI ANA Kit is an indirect immunofluorescence assay for the detection and semi-quantitative determination of anti-nuclear antibodies in human serum.

Kit components:

- HEp-2 (human epithelial cell) substrate slides; 12 wells/slide, with desiccant
- FITC IgG Conjugate with 4',6-diamidino-2-phenylindole (DAPI), containing 0.09% sodium azide; ready to use
- Positive Control: ANA Titratable Pattern, human serum with antibodies to HEp-2 nuclei in buffer, containing 0.09% sodium azide; pre-diluted, ready to use
- Negative Control: IFA System Negative Control, diluted human serum with no ANA

- present, containing 0.09% sodium azide; pre-diluted, ready to use
- PBS II (40x) Concentrate, sufficient for making 2000 mL of 1x PBS II
- Mounting Medium, containing 0.09% sodium azide
- Coverslips

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

NOVA Lite® HEp-2 ANA kit

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

k880736

3. Comparison with predicate:

<b>Similarities</b>		
<b>Item</b>	<b>Device: NOVA Lite® DAPI ANA Kit</b>	<b>Predicate: NOVA Lite® HEp-2 ANA Kit</b>
Intended Use	Qualitative or semi-quantitative detection of anti-nuclear antibodies (ANA) used as an aid in the diagnosis of SLE and other systemic rheumatic diseases in conjunction with other clinical and laboratory findings.	Same
Methodology	Indirect Immunofluorescence (IIF)	Same
Procedure	Standard IIF technique	Same
Reported Result	Qualitative, Semi-quantitative titer	Same
Sample Matrix	Serum	Same
Analyte	ANA of IgG isotype	Same
Antigen	HEp-2 cells	Same
Slides	12-well coated with antigen	Same
Conjugate	FITC-conjugated anti-human IgG (Fc specific)	Same
Controls	One positive (homogenous pattern) control and one negative control	Same
Storage	2–8°C	Same

<b>Differences</b>		
<b>Item</b>	<b>Device: NOVA Lite® DAPI ANA Kit</b>	<b>Predicate: NOVA Lite® HEp-2 ANA kit</b>
Recommended sample dilution	1:80	1:40
Cut-off Level	1:80	1:40
Counterstain/additional dye in conjugate	4',6-diamidino-2-phenylindole (DAPI)	None
Interpretation	Manual fluorescence microscopy or NOVA View Device	Manual fluorescence microscopy
Shelf-life Stability	12 months	24 months

**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).

C28-A3, Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory; Approved Guideline, Third Addition.

EP07-A2, Interference Testing in Clinical Chemistry, Approved Guideline, Second Edition.

EP09-A2IR, Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, Second Edition (Interim Revision) (used for matrix comparison).

**L. Test Principle:**

Samples diluted 1:80 in PBS are incubated with the antigen substrate (HEp-2 cells) coated on the slide. After incubation, unbound antibodies are washed off. The substrate is then incubated with anti-human IgG-FITC conjugate. The conjugate contains a DNA-binding blue fluorescent dye, 4',6-diamidino-2-phenylindole (DAPI) that is required for NOVA View® use. Unbound reagent is washed off, and the slides are coverslipped. Stained slides can be read by manual fluorescence microscopy, or scanned with the NOVA View®. The NOVA View® will suggest a result (positive/negative) and a pattern (homogeneous, speckled, centromere, nucleolar, nuclear dots or unrecognized) for positive results. The resulting digital images can be reviewed and interpreted from the computer monitor. Samples that are positive at 1:80 may be titered manually by performing a two-fold serial dilution from the initial screening dilution with PBS buffer (i.e. 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, etc.) to determine the endpoint titer.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

Nomenclature used in studies:

- Throughout the submission, results obtained by manual reading of the same slides are used as reference method
- “Manual” and “Manual reading” refer to results obtained by the operator reading and interpreting the slides with a traditional fluorescence microscope.
- “Digital”, “Digital reading” and “Digital image” refers to results obtained by the operator reading NOVA View® generated images on the computer monitor, blinded to the suggested interpretation.
- “NOVA View®” refers to results obtained with the NOVA View® Automated Fluorescent Microscope, such as Light Intensity Units (LIU), positive/negative classification and pattern information.

a. *Precision/Reproducibility:*

*Repeatability*

To assess repeatability of the NOVA Lite® DAPI ANA kit using the NOVA View® and a manual microscope, three different studies were performed. For each study, samples were diluted for each run separately; therefore, if 10 runs were performed, 10 dilutions were prepared at the beginning of the slide processing. Within one run, the same dilution was tested in triplicate. The same reagent lot was used by all three repeatability studies.

The first study tested three negative and 10 positive samples with various patterns and intensities were stained with NOVA Lite® DAPI ANA kit, and tested in triplicates in 10 runs (two runs per day), resulting in 30 data points for each sample. The slides were scanned by NOVA View®, and the resulting digital images were interpreted by the operator. The slides in this study were not read with a manual microscope; i.e. only two set of results were generated: NOVA View® output and digital image reading results. The percentage of positive/negative calls are presented below:

Sample ID	N	NOVA View output			Digital Reading	
		Mean LIU	% Negative	% Positive	% Negative	% Positive
NVB012	30	4.7	100%	0%	100%	0%
NVB007	30	7.6	100%	0%	100%	0%
NVB063	30	7.9	100%	0%	100%	0%
NVB111	30	38.5	63.3%	36.7%	3.3%	96.7%
NVB079	30	91.6	13.3%	86.7%	3.3%	96.7%
NVB009	30	229.1	0%	100%	0%	100%

NVB029	30	233.8	0%	100%	0%	100%
NVB017	30	310.5	0%	100%	0%	100%
NVB087	30	310.6	0%	100%	0%	100%
NVB023	30	715.5	0%	100%	0%	100%
NVB004	30	933.3	0%	100%	0%	100%
NVB118	30	1300.1	0%	100%	0%	100%
NVB037	30	2217.7	0%	100%	0%	100%

A second study cohort of samples was selected to challenge the cut-off LIU of the NOVA View® System. Twenty-two samples covering all patterns identified by the NOVA View® which included 20 samples considered borderline/LIU values around cut-off, and 2 samples with 3+ average grade intensity level. Samples were tested in three replicates, in 10 runs (2 runs per day), resulting in 30 data points for each sample. Samples were diluted to target low-positive samples and challenge the NOVA View® LIU and then diluted a second time at 1:80 per the kit instructions for use. The slides were scanned with NOVA View®, and digital images were interpreted. The same slides were then read with a manual microscope. In total, three set of results were generated: NOVA View® output, digital image reading results and manual reading results.

Sample ID	N	NOVA View output			Manual Reading		Digital Reading	
		Mean LIU	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
NV20	30	3.5	100%	0%	100%	0%	100%	0%
NV16	30	10.2	100%	0%	100%	0%	100%	0%
NV2	30	11.4	100%	0%	100%	0%	100%	0%
NV8	30	13.5	100%	0%	100%	0%	100%	0%
NV15	30	16.6	100%	0%	13.3%	86.7%	16.7%	83.3%
NV9	30	19.1	100%	0%	46.7%	53.3%	100%	0%
SB24216	30	31.4	86.7%	13.3%	0%	100%	0%	100%
NV22	30	33.6	76.7%	23.3%	96.7%	3.3%	76.7%	23.3%
NV26	30	38.4	90.0%	10.0%	60.0%	40.0%	53.3%	46.7%
NV14	30	38.8	43.3%	56.7%	6.7%	93.3%	0%	100%
NV13	30	40.5	66.7%	33.3%	0%	100%	6.7%	93.3%
NV5	30	40.7	66.7%	33.3%	0%	100%	16.7%	83.3%
NVB440	30	43.8	73.3%	26.7%	33.3%	66.7%	46.7%	53.3%
NV4	30	57.5	43.3%	56.7%	0%	100%	0%	100%
NVB201	30	62.8	26.7%	73.3%	0%	100%	0%	100%
NVB074	30	63.8	16.7%	83.3%	0%	100%	0%	100%
NV12	30	64.8	36.7%	63.3%	0%	100%	0%	100%
NVB369	30	72.4	23.3%	76.7%	3.3%	96.7%	13.3%	86.7%
NV7	30	74.1	10.0%	90.0%	0%	100%	0%	100%
NV10	30	128.5	30.0%	70.0%	0%	100%	0%	100%
NV23	30	822.4	0%	100%	0%	100%	0%	100%
NV6	30	903.9	0%	100%	0%	100%	0%	100%

A third, separate study was also performed with samples tested in triplicates or duplicates, in five runs, resulting in 15 or 10 data points for each sample. The slides were scanned with the NOVA View®, and digital images were interpreted. Slides were also read with a manual microscope. Three set of results were generated: NOVA View® output, digital image reading results and manual reading results.

Sample ID	N	NOVA View output			Manual Reading		Digital Reading	
		Mean LIU	% Negative	% Positive	% Negative	% Positive	% Negative	% Positive
PMDx 5087	15	24.6	100%	0%	100%	0%	100%	0%
SS-A Monospecific 08203	15	103.6	0%	100%	0%	100%	0%	100%
AMA 930328	15	882.6	0%	100%	0%	100%	0%	100%
Centromere 120571	10	1052.9	0%	100%	0%	100%	0%	100%
Nucleolar 120559	10	1339.8	0%	100%	0%	100%	0%	100%
DNA PS0007 520847	15	1375.6	0%	100%	0%	100%	0%	100%
ANA DNA 420530	10	1607.8	0%	100%	0%	100%	0%	100%
SmRNP 220951	10	2811.2	0%	100%	0%	100%	0%	100%

For both digital image reading and manual reading for study one and study two, intensity grades were within  $\pm 1$  reactivity grade within one run (within triplicates), and the average grade was no more than one reactivity grade different between runs. Pattern determination was consistent for 100% of the replicates (for positive samples only).

*Reproducibility:*

To assess between operator and between instrument variability, a reproducibility study was performed at Inova Diagnostics (internal; Site#1) and at two external sites (Sites #2, #3) using the same sample cohort.

A cohort of 120 samples at each location was processed with NOVA Lite® DAPI ANA kit, and scanned with NOVA View®. Digital images were interpreted and confirmed. Additionally, a second operator read and interpreted the same digital images at each location. Altogether, six digital image datasets were generated (three locations, two operators at each site). The same digital images were read by the two operators at each site, but different slides were read at all three locations.

The 120 samples were selected to represent approximately 50% negative and 50% positive samples with various patterns. All major patterns were represented, and reactivity grades ranged from 0 to 4.

Within-Site Reproducibility:

Within-Site Agreement:

N=120		Positive Agreement % (95% CI)	Negative Agreement % (95% CI)	Total Agreement % (95% CI)
Site#1	NOVA View vs Manual	100.0 (93.7–100.0)	98.4 (91.5–100.0)	99.2 (95.4–100.0)
	Digital vs Manual	100.0 (93.7–100.0)	98.4 (91.5–100.0)	99.2 (95.4–100.0)
	Digital vs NOVA View	100.0 (93.8–100.0)	100.0 (94.2–100.0)	100.0 (97.0–100.0)
Site#2	NOVA View vs Manual	95.0 (81.6–99.0)	98.3 (91.1–100.0)	96.7 (91.7–99.1)
	Digital vs Manual	96.7 (88.5–99.6)	95.0 (86.1–99.0)	95.8 (90.5–98.6)
	Digital vs NOVA View	93.4 (84.1–98.2)	98.3 (90.9–100.0)	95.8 (90.5–98.6)
Site#3	NOVA View vs Manual	94.6 (85.1–96.8)	98.4 (91.6–100.0)	96.7 (91.7–99.1)
	Digital vs Manual	92.9 (82.7–98.0)	100.0 (94.4–100.0)	96.7 (91.7–99.1)
	Digital vs NOVA View	100.0 (93.2–100.0)	97.1 (89.9–99.6)	98.3 (94.1–99.8)

Within-Site Pattern Agreement across method:

Pattern agreement was assessed in pair-wise comparison between manual reading, NOVA View® results, and digital image reading at each site. Only definitive patterns (Homogeneous, Speckled, Centromere, Nucleolar, Nuclear dots) were considered as pattern agreement. NOVA View® reported “Unrecognized” patterns and user reported “Other” patterns were not considered as an agreement.

Out of the 120 samples in the reproducibility cohort, there were 57 positive samples at Site #1, 60 at Site #2 and 56 at Site #3 by manual reading (reference method). A summary table of pattern agreement is shown below:

N=120	Number (%) of samples with pattern agreement*		
	Site #1	Site #2	Site #3
Digital vs Manual	96.5%	95.0%	96.4%
NOVA View vs Manual	78.9%	83.3%	80.4%
Digital vs NOVA View	77.2%	80.0%	80.4%

\*As percentage of samples that were positive with manual interpretation.

Fluorescent intensity (grade) agreement:

Fluorescence intensity grades were within  $\pm$  one grade from each other between manual reading and digital image reading, as shown below:

N=120	Percent of samples within $\pm$ one grade		
	Site #1	Site #2	Site #3
Digital vs Manual	98.3%	99.2%	99.2%

Between-Site Reproducibility:

Between-site reproducibility was assessed by calculating average positive, average negative and total agreement between NOVA View® generated results, digital image reading result and manual (traditional) reading results between the three sites. Confidence intervals were determined using bootstrap analysis. Results are shown below:

Manual Reading Between-Site:

Manual Reading N=120	Site #1 vs. Site #2	Site #1 vs. Site #3	Site #2 vs. Site #3
Average Positive Agreement % (95%CI)	97.4 (94.0–100.0)	99.1 (97.0–100.0)	96.6 (92.7–99.2)
Average Negative Agreement % (95%CI)	97.6 (94.3–100.0)	99.2 (97.4–100.0)	96.8 (93.1–99.3)
Overall Agreement % (95%CI)	97.5 (92.9–99.5)	99.2 (95.4–100.0)	96.9 (91.7–99.1)

Digital Reading Between-Site:

Digital Reading* N=120	Site #1 vs. Site #2	Site #1 vs. Site #3	Site #2 vs. Site #3
Average Positive Agreement % (95%CI)	95.8 (91.6–99.2)	94.5 (89.6–98.3)	92.0 (86.2–96.7)
Average Negative Agreement % (95%CI)	95.9 (97.1–99.2)	95.4 (91.2–98.6)	92.9 (87.7–97.1)
Overall Agreement % (95%CI)	95.8 (90.5–98.6)	95.0 (89.4–98.1)	92.5 (86.2–96.5)

\*Considering only Operator #1 results. Operator #2 had similar results (see between-operator agreement below).

NOVA View® Interpretation Between-Site:

NOVA View N=120	Site #1 vs. Site #2	Site #1 vs. Site #3	Site #2 vs. Site #3
Average Positive Agreement % (95%CI)	100.0 (100.0–100.0)	96.4 (92.4–99.2)	96.4 (92.4–99.2)
Average Negative Agreement % (95%CI)	100.0 (100.0–100.0)	96.6 (93.3–99.3)	96.6 (93.3–99.3)
Overall Agreement % (95%CI)	100.0 (97.0–100.0)	96.7 (91.7–99.1)	96.7 (91.7–99.1)

*Between Operator Agreement:*

Between operators, total agreement was > 90 % in each of the 15 pair-wise comparisons, as shown in the matrix below:

% Overall Agreement (Positive/Negative) for digital image reading across all operators						
	Site #1 Op #1	Site #1 Op #2	Site #2 Op #1	Site #2 Op #2	Site #3 Op #1	Site #3 Op #2
Site #1 Op #1	N/A	99.2%	95.8%	100.0%	95.0%	94.2%
Site #1 Op #2		N/A	95.0%	99.2%	94.2%	93.3%
Site #2 Op #1			N/A	95.8%	92.5%	91.7%
Site #2 Op #2				N/A	95.0%	94.2%
Site #3 Op #1					N/A	99.2%

*Lot-to-Lot Comparison*

A lot-to-lot reproducibility study was also performed using three reagent lots: 008559, 009398 and 009399. Forty sera were tested along with eight controls (one negative and one positive control on each slide).

The following comparisons were made:

- Digital image reading: negative/positive, grade and pattern comparison
- Manual image reading: negative/positive, grade and pattern comparison
- NOVA view® output: negative/positive, LIU and pattern comparison

Digital Reading Between-Lot Agreement:

Digital Reading	Lot 008559 vs Lot 009398	Lot 008559 vs Lot 009399	Lot 009398 vs Lot 009399
Average Positive Agreement % (95%CI)	95.2 (87.2–100.0)	93.0 (83.3–100.0)	97.6 (90.9–100.0)
Average Negative Agreement % (95%CI)	94.7 (85.7–100.0)	91.9 (80.9–100.0)	97.4 (90.9–100.0)
Overall Agreement % (95%CI)	95.0 (83.1–99.4)	92.5 (79.6–94.4)	97.5 (86.8–99.9)

Manual Reading Between-Lot Agreement:

Digital Reading	Lot 008559 vs Lot 009398	Lot 008559 vs Lot 009399	Lot 009398 vs Lot 009399
Average Positive Agreement % (95%CI)	95.8 (88.9–100.0)	95.8 (88.9–100.0)	100.0 (100.0–100.0)
Average Negative Agreement % (95%CI)	93.8 (82.8–100.0)	93.8 (82.8–100.0)	100.0 (100.0–100.0)
Overall Agreement % (95%CI)	95.0 (83.1–99.4)	95.0 (83.1–99.4)	100.0 (91.2–100.0)

*Lot-to-Lot Grade Agreement:*

All grades (100%) were within  $\pm 1$  grade from each other for all samples in any pair-wise comparisons.

*Lot-to-Lot Pattern Agreement:*

Pattern agreement was assessed in pair-wise comparison between lots. Only definitive patterns (Homogeneous, Speckled, Centromere, Nucleolar, Nuclear dots) were considered as pattern agreement. User reported “Other” patterns were not considered as an agreement. There was 100% pattern agreement between the lots with manual and digital (not NOVA View only) image reading.

*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.

Stability:

Accelerated stability study was performed on three lots of conjugate with DAPI according to an isochronous design, at  $37 \pm 3^\circ\text{C}$  for 4 weeks. Each week a new vial of sealed component was placed in the incubator at  $37 \pm 3^\circ\text{C}$ , and all components were tested at the end of the study period together with a vial that was stored at  $5 \pm 3^\circ\text{C}$  (control). Testing was performed by staining the slides with characterized samples. All calculations were performed by comparing results obtained with the control vial (stored at  $5 \pm 3^\circ\text{C}$ ) to those obtained with vials stored at  $37 \pm 3^\circ\text{C}$  for 1, 2, 3, and 4 weeks, where one week is equal to six months at  $5 \pm 3^\circ\text{C}$ . All slides were

scanned with NOVA View® and with manual microscopy by the same operator.

The sponsor's acceptance criteria for one year preliminary shelf life was that reactivity grades obtained on slides stored at 37 °C for 2 weeks were within ± one grade of those obtained on the control slides. All criteria were met.

Closed vial stability claim is one year at 5 ± 3°C.

*d. Detection limit:*

Not applicable

*e. Analytical specificity:*

Interference:

The interference study was performed according to CLSI EP07-A2. Interference by bilirubin, hemoglobin, triglycerides, cholesterol and rheumatoid factor (RF) IgM was assessed using the following final three testing concentrations:

Interfering substance	Maximum final Concentration tested	Medium final Concentration tested	Minimum final concentration tested
Bilirubin, conjugated	10 mg/dL	5 mg/dL	2.5 mg/dL
Hemoglobin	200 mg/dL	100 mg/dL	50 mg/dL
Triglycerides	1000 mg/dL	500 mg/dL	250 mg/dL
Cholesterol	224.3 mg/dL	112.1 mg/dL	56 mg/mL
RF IgM	56 AU	33.6 AU	11.2 AU

Three specimens were tested (one negative, one medium positive, and one strong positive) in triplicate for each level of interferant. Interfering substances (hemoglobin, bilirubin, triglycerides, and cholesterol) were spiked into every specimen at three different concentrations in 10% of total specimen volume resulting in the final interferant concentrations indicated in the table above. To assess interference with rheumatoid factor (RF), 10%, 30% and 50% RF positive sample by volume was added to the test samples. Appropriate controls were made by adding 10% sample diluent to the same samples. All samples were processed with NOVA Lite® DAPI ANA kit, and scanned with NOVA View®. Digital images were interpreted and confirmed. All slides were read by the same operator with manual microscopy.

Reactivity grades of samples containing the interfering substance were within ± one grade of the control samples with both manual and digital reading.

No interference was detected with bilirubin up to 10 mg/dL, hemoglobin up to 200

mg/dL, triglycerides up to 1000 mg/dL, cholesterol up to 224.3 mg/dL and RF IgM up to 56 AU.

Cross-reactivity:

Refer to clinical study section and differential diagnosis samples.

The CDC ANA reference standards (also known as IUIS ANA reference standards) were tested with the NOVA Lite® DAPI ANA kit, and scanned with NOVA View®. Digital images were interpreted and confirmed. Additionally, slides were read with traditional manual microscope by the same operator.

All reference sera produced the expected pattern. The results of NOVA View® digital image trained operator confirmed interpretation were within  $\pm$  one reactivity grade from that of manual interpretation of the slides. No discrepancies in pattern interpretation were seen between manual and digital results.

CDC Reference Serum ID	Expected ANA pattern	Known antibody specificity	Pattern with Manual microscopic reading	Pattern with Digital image reading
ANA Human Reference Serum #1	Homogeneous/ Rim	nDNA	Homogeneous	Homogeneous
ANA Human Reference Serum #2	Speckled	SS-B/La	Speckled	Speckled
ANA Human Reference Serum #3	Speckled	RNP, SS-B/La, SS-A/Ro	Speckled	Speckled
ANA Human Reference Serum #4	Speckled	U1-RNP	Speckled	Speckled
ANA Human Reference Serum #5	Speckled	Sm	Speckled	Speckled
ANA Human Reference Serum #6	Nucleolar	Fibrillarin	Nucleolar	Nucleolar
ANA Human Reference Serum #7	N/A	SS-A/Ro	Speckled	Speckled
ANA Human Reference Serum #8	Centromere	Centromere	Centromere	Centromere

CDC Reference Serum ID	Expected ANA pattern	Known antibody specificity	Pattern with Manual microscopic reading	Pattern with Digital image reading
ANA Human Reference Serum #9	N/A	Scl-70	Homogeneous	Homogeneous
ANA Human Reference Serum #10	N/A	Jo-1	ANA Negative; Cytoplasmic speckled (Jo-1 like)	ANA Negative; Cytoplasmic speckled (Jo-1 like)
ANA Human Reference Serum #11	N/A	PM-Scl	Nucleolar	Nucleolar
ANA Human Reference Serum #12	N/A	Ribosomal P	Negative*	Negative*

\*Anti-ribosomal antibodies show variable levels of detectability on HEp-2 cells

*f. Assay cut-off:*

The recommended starting dilution, above which the result is reported as positive and below which the result is reported as negative, is 1:80 for use with the NOVA View® System and manual microscopy. The serum dilution of 1:80 was selected to provide optimal clinical sensitivity and specificity.

Light Intensity Unit (LIU) Cut-off:

The cut-off LIU has been established on 120 serum samples from apparently healthy blood bank donors. All samples were processed with NOVA Lite® DAPI ANA kit, and scanned with NOVA View®. Digital images were interpreted and confirmed. All slides were read by the same operator with manual microscopy.

The specificity value for the manual read was used as reference to establish the cut-off in LIU values with the performance goal that the NOVA View® report results showing good agreement with results generated by manual reading.

The LIU values for the 120 samples ranged from 0 to 286 LIU, with a mean value of 20 and SD of 34. The cut-off was established with the non-parametric percentile method. The 90th percentile of LIU values was 48.8 (90% CI: 31 to 78%), so 48 LIU was selected as the cut-off LIU. The cut-off value is entered in the software during NOVA View® installation and setup, and is used for classification of samples as negative or positive. The cut-off for the NOVA View® cannot be modified.

At a cut-off level of 48 LIU, NOVA View® classification showed 98.3% overall

agreement with digital image reading results, and 95.0% overall agreement with manual reading results. The overall agreement between digital image reading and manual reading was determined to be 96.7%.

2. Comparison studies:

a. *Method comparison with predicate device:*

To demonstrate the equivalent performance of the NOVA Lite® DAPI ANA Kit which contains the DAPI conjugate and is used with a cut-off of 1:80 to the predicate kit without DAPI and a 1:40 starting dilution, a method comparison study was performed using 410 samples made up of 400 clinically characterized sera, and 10 samples with known ANA patterns.

All slides were interpreted with traditional fluorescence microscopy only. Interpretation included positive/negative categorization, pattern interpretation and grading of positive samples on a scale of 1+ to 4+.

The distribution of the cohort and the frequency of positive results are shown in the table below:

Disease status	# of samples	New kit: NOVA Lite DAPI ANA Kit 1:80		Predicate: NOVA Lite HEp-2 ANA Kit 1:40	
		# pos	% pos	# pos	% pos
Healthy controls	150	17	11.3%	41	27.3%
SLE	100	80	80%	85	85%
Sjögren's (SS)	30	21	70%	23	76.7%
Systemic sclerosis (SSc)	30	15	50%	20	66.7%
Autoimmune myositis (AIM)	10	7	70%	9	90%
MCTD	20	12	60%	12	60%
Infectious disease	30	4	13.3%	5	16.7%
Rheumatoid arthritis (RA)	30	17	56.7%	20	66.7%
Centromere Ab Pos	5	5	100%	5	100%
Mitochondrial Ab Pos	5	5	100%	4	80%
Total	410	183		224	

*Agreement between methods:*

1:80 vs 1:40 Dilution		Predicate NOVA Lite HEp-2 ANA Kit, 1:40		
		Positive	Negative	Total
New Assay NOVA Lite DAPI ANA Kit, 1:80	Positive	179	5	184
	Negative	45	181	226
	Total	224	186	410

Positive Agreement = 79.9% (179/224) (95% CI: 74.1 – 85.0%)

Negative Agreement = 97.3% (181/186) (95% CI: 91.5 – 100.0%)

Overall Agreement = 87.7% (301/410) (95% CI: 84.2 – 90.8%)

*Pattern Agreement:*

A total of 179 samples were positive by both kits/dilutions. There was 97% agreement between the two dilutions. Of the positive samples, there were only five discrepant including patterns interpreted as “other”.

*Grade Agreement:*

Fluorescence intensity grades were within  $\pm$  one grade from each other for 407 samples (99.5%). Grade agreement is shown in the matrix below:

		Fluorescence grade, predicate device 1:40					
		0	1+	2+	3+	4+	Total
Fluorescence grade NOVA Lite DAPI ANA Kit, 1:80	0	181	44	1	0	0	226
	1+	3	35	31	0	0	69
	2+	1	3	37	24	0	65
	3+	0	0	0	14	11	25
	4+	0	0	0	1	23	24
	Total	185	82	69	39	34	409*

\*Grade was not reported for one sample

*Clinical Sensitivity and Specificity for cohort:*

	Sensitivity % (95% CI)		Specificity** % (95% CI) N= 60
	SLE N=100	SARD* N=190	
New Assay: NOVA Lite DAPI ANA Kit 1:80	80.8% (71.7 – 88.0)	71.4% (64.4 – 77.8)	65.0% (51.6 – 76.9)
Predicate NOVA Lite HEp-2 ANA Kit 1:40	85.9% (77.4 – 92.0)	78.8% (72.3 – 84.4)	56.7% (43.2 – 69.4)

\*SARD: Systemic Autoimmune Rheumatic Disease (includes SLE, SSc, SS, MCTD and IIM)

\*\*Control samples include RA and infectious disease population

*Conjugate comparison:*

The NOVA Lite® DAPI ANA Kit contains the same components as the predicate device, with the exception of the conjugate. To adapt the assay for use on NOVA View®, the blue fluorescent dye DAPI (4',6-diamidino-2-phenylindole) that binds strongly to A-T rich regions in DNA was added to the conjugate. The addition of DAPI does not influence the test utility and performance when used manually, as it is not visible at the wavelength used for reading slides with traditional fluorescence microscopy, and does not interfere with antibody binding.

To demonstrate the equivalent performance of the conjugate with and without DAPI, a comparison study has been performed on clinical samples.

407 individual serum samples have been tested. Two sets of slides were stained: one with the conjugate without DAPI (the predicate device), the other with the conjugate with DAPI (NOVA Lite® DAPI ANA Kit). The 1:80 serum dilution was used for both kits. The two sets of slides were read by the same operator by manual microscopy. Positive/negative agreement, pattern agreement and grade correlation were evaluated.

*Agreement:*

DAPI Comparison		Predicate Conjugate without DAPI		
		Positive	Negative	Total
New Assay Conjugate With DAPI	Positive	210	11	221
	Negative	3	183	186
	Total	213	194	407

Positive Agreement = 98.6% (210/213) (95% CI: 95.9 – 99.7%)

Negative Agreement = 94.3% (183/194) (95% CI: 90.1 – 97.1%)

Overall Agreement = 96.6% (393/407) (95% CI: 94.3 – 98.1%)

*Grade Agreement:*

		Predicate Conjugate without DAPI					
		0	1+	2+	3+	4+	Total
New Assay Conjugate With DAPI	0	183	3	0	0	0	186
	1+	11	71	8	0	0	90
	2+	0	14	73	3	0	90
	3+	0	0	2	26	2	30
	4+	0	0	0	2	9	11
	Total	194	88	83	31	11	407

b. *Matrix comparison:*

Not applicable

3. Clinical studies:

a. *Clinical Sensitivity and Specificity:*

To determine accuracy and clinical sensitivity and specificity, a cohort of 463 clinically characterized samples were processed with NOVA Lite® DAPI ANA kit, and scanned with NOVA View®. Digital images were independently interpreted and confirmed by a trained operator. Additionally, slides were read with traditional manual microscope by the same operator. The same slides were read at three different locations, one internal (site 1) and two external (sites 2 and 3).

The number and distribution of the samples are shown below:

Sample type	Number of samples
Healthy control	75
HBV	20
HCV	5
HIV	5
Syphilis	5
Systemic Lupus Erythematosus (SLE)	75
Systemic Sclerosis (SSc)	20
Sjögren's syndrome(SS)	20
Autoimmune Liver Disease (AIL)	20
Rheumatoid arthritis (RA)	20
Mixed Connective Tissue Disease (MCTD)	21
Autoimmune myositis	26
Fibromyalgia	25
Anti-MPO/anti-PR3	26
Crohn's/Inflammatory bowel disease	20
Autoimmune thyroiditis	24
Celiac disease	24
Drug induced lupus (DIL)	25
Other	7
Total	463

Number Positive and Percent Positivity rates in the various disease cohorts by method (NOVA View®, Manual read or Digital read) at the three locations are listed below:

		Number of positive samples								
		Manual			Digital			NOVA View		
Sample type	N	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Healthy control	75	4	7	13	5	2	8	4	2	19
HBV	20	5	4	2	3	3	1	1	1	4
HCV	5	0	1	2	2	1	2	2	1	2
HIV	5	0	0	2	2	1	2	2	2	5
Syphilis	5	0	0	3	3	0	3	3	1	3
SLE	75	54	53	62	60	55	61	60	54	62
SSc	20	19	19	19	19	19	19	19	19	19
SS	20	9	11	14	13	9	14	12	9	15
AIL	20	16	18	17	20	17	18	20	17	20
RA	20	11	15	15	14	14	13	15	13	13
MCTD	21	10	10	8	10	8	8	10	8	8
Autoimmune myositis	26	7	9	10	6	7	7	6	8	8
Fibromyalgia	25	9	11	9	6	6	10	6	5	8
Anti-MPO/anti-PR3	26	3	5	4	4	4	4	1	5	5
Crohn's/Inflammatory bowel disease	20	9	8	8	8	7	7	8	6	7
Autoimmune thyroiditis	24	4	6	5	3	2	4	2	3	5
Celiac disease	24	4	7	7	3	5	4	3	3	2
Drug induced lupus	25	5	5	7	5	5	5	4	5	4
Other	7	2	1	2	1	1	1	1	1	2
Total	463	171	190	209	187	166	191	179	163	211

		Percent Positive Samples								
		Manual			Digital			NOVA View		
Sample type	N	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3	Site 1	Site 2	Site 3
Healthy control	75	5.3%	9.3%	17.3%	6.7%	2.7%	10.7%	5.3%	2.7%	25.3%
HBV	20	25.0%	20.0%	10.0%	15.0%	15.0%	5.0%	5.0%	5.0%	20.0%
HCV	5	0.0%	20.0%	40.0%	40.0%	20.0%	40.0%	40.0%	20.0%	40.0%
HIV	5	0.0%	0.0%	40.0%	40.0%	20.0%	40.0%	40.0%	40.0%	100.0%
Syphilis	5	0.0%	0.0%	60.0%	60.0%	0.0%	60.0%	60.0%	20.0%	60.0%
SLE	75	72.0%	70.7%	82.7%	80.0%	73.3%	81.3%	80.0%	72.0%	82.7%
SSc	20	95.0%	95.0%	95.0%	95.0%	95.0%	95.0%	95.0%	95.0%	95.0%
SS	20	45.0%	55.0%	70.0%	65.0%	45.0%	70.0%	60.0%	45.0%	75.0%
AIL	20	80.0%	90.0%	85.0%	100.0%	85.0%	90.0%	100.0%	85.0%	100.0%
RA	20	55.0%	75.0%	75.0%	70.0%	70.0%	65.0%	75.0%	65.0%	65.0%
MCTD	21	47.6%	47.6%	38.1%	47.6%	38.1%	38.1%	47.6%	38.1%	38.1%
Autoimmune myositis	26	26.9%	34.6%	38.5%	23.1%	26.9%	26.9%	23.1%	30.8%	30.8%
Fibromyalgia	25	36.0%	44.0%	36.0%	24.0%	24.0%	40.0%	24.0%	20.0%	32.0%
Anti-MPO/ anti-PR3	26	11.5%	19.2%	15.4%	15.4%	15.4%	15.4%	3.8%	19.2%	19.2%
Crohn's/ Inflammatory bowel disease	20	45.0%	40.0%	40.0%	40.0%	35.0%	35.0%	40.0%	30.0%	35.0%
Autoimmune thyroiditis	24	16.7%	25.0%	20.8%	12.5%	8.3%	16.7%	8.3%	12.5%	20.8%
Celiac disease	24	16.7%	29.2%	29.2%	12.5%	20.8%	16.7%	12.5%	12.5%	8.3%
Drug induced lupus (DIL)	25	20.0%	20.0%	28.0%	20.0%	20.0%	20.0%	16.0%	20.0%	16.0%
Other	7	28.6%	14.3%	28.6%	14.3%	14.3%	14.3%	14.3%	14.3%	28.6%
Total	463									

Because of concerns about sample quality, 21 of the 25 DIL samples have not been included in the sensitivity calculations, but were included in the agreement calculations. The remaining four DIL samples were included in the sensitivity calculations.

Sensitivity was calculated at each site for SLE separately, and on the combination of the connective tissue diseases (CTD) (SLE + systemic sclerosis + Sjögren’s + MCTD + autoimmune myositis + DIL) plus autoimmune liver disease (AIL) population. Specificity was calculated on the total control population excluding healthy subjects.

Site 1:

Site 1	Sensitivity % (95% CI)		Specificity % (95% CI) no healthy N= 174
	SLE N=75	CTD+AIL N=186	
Manual Read	72.0 (60.4 to 81.8)	62.9 (55.5 to 69.9)	74.1 (67.0 to 80.5)
Digital Read	80.0 (69.2 to 88.4)	69.9 (62.8 to 76.4)	72.4 (65.1 to 78.9)
NOVA View	80.0 (69.2 to 88.4)	69.4 (62.2 to 75.9)	75.3 (68.2 to 81.5)

Site 2:

Site 2	Sensitivity % (95% CI)		Specificity% (95% CI) no healthy N= 174
	SLE N=75	CTD+AIL N=186	
Manual Read	70.7 (59.0 to 80.6)	65.6 (58.3 to 72.4)	67.2 (59.7 to 74.2)
Digital Read	73.3 (61.9 to 82.9)	62.9 (55.5 to 69.9)	75.3 (68.2 to 81.5)
NOVA View	72.0 (60.4 to 81.8)	62.9 (55.5 to 69.9)	77.0 (70.0 to 83.0)

Site 3:

Site 3	Sensitivity % (95% CI)		Specificity % (95% CI) no healthy N= 174
	SLE N=75	CTD+AIL N=186	
Manual Read	82.7 (72.2 to 90.4)	71.0 (63.9 to 77.4)	67.2 (59.7 to 74.2)
Digital Read	81.3 (70.7 to 89.4)	69.4 (62.2 to 75.9)	71.3 (63.9 to 77.9)
NOVA View	82.7 (72.2 to 90.4)	72.0 (65.0 to 78.4)	69.0 (61.5 to 75.7)

Agreement results between NOVA View® classification, digital image reading and manual reading were calculated within each testing location and between locations:

Within-Site Agreement:

N=463		Positive Agreement % (95% CI)	Negative Agreement % (95% CI)	Total Agreement % (95% CI)
Site#1	NOVA View vs. Manual	88.3 (82.5–92.7)	90.4 (86.4–93.5)	89.6 (86.5–92.3)
	Digital vs. Manual	93.0 (88.1–96.3)	90.4 (86.4–93.5)	91.4 (88.4–93.8)
	NOVA View vs. Manual	94.1 (89.7–97.0)	98.9 (96.9–99.8)	97.0 (95.0–98.3)
Site#2	NOVA View vs. Manual	80.5 (74.2–85.9)	96.3 (93.4–98.2)	89.8 (86.7–92.4)
	Digital vs. Manual	84.2 (78.2–89.1)	97.8 (95.3–99.2)	92.2 (89.4–94.5)
	Digital vs NOVA View	94.0 (89.2–97.1)	97.6 (95.2–99.0)	96.3 (94.2–97.8)
Site#3	NOVA View vs. Manual	86.1 (80.7–90.5)	87.8 (83.1–91.6)	87.0 (83.6–90.0)
	Digital vs. Manual	87.1 (81.8–91.3)	96.5 (93.4–98.4)	92.2 (89.4–94.5)
	Digital vs NOVA View	95.8 (91.9– 98.2)	89.7 (85.5–93.0)	92.2 (89.4–94.5)

Between Site Overall Agreement by interpretation method:

Between Site Agreement N = 463		
Manual	Site#1 Manual	Site#2 Manual
Site#2 Manual	90.7 (87.7–93.2)	
Site#3 Manual	85.7 (82.2–88.8)	87.3 (83.9–90.2)
Digital	Site#1 Digital	Site#2 Digital
Site#2 Digital	92.0 (89.2–94.3)	
Site#3 Digital	93.1 (90.4–95.2)	92.0 (89.2–94.3)
NOVA View	Site#1 NOVA View	Site#2 NOVA View
Site#2 NOVA View	92.7 (89.9–94.9)	
Site#3 NOVA View	89.6 (86.5–92.3)	87.9 (84.6–90.7)

**Within-Site Pattern Agreement:**

Pattern agreement was assessed in pair-wise comparison between manual reading, NOVA View® results, and digital image reading. Only definitive patterns (homogeneous, speckled, centromere, nucleolar, nuclear dots) were considered as pattern agreement. NOVA View® reported “Unrecognized” patterns and user reported “Other” patterns were not considered as an agreement.

Out of the 463 clinical samples, there were 171 positive samples at Site #1, 190 at Site #2 and 209 at Site #3 by manual reading (reference method). Agreement between digital image reading and manual reading was above 90% at all three testing sites.

Summary table of pattern percent agreement is shown below:

N=463	Number (%) of samples with pattern agreement*		
	Site#1	Site#2	Site#3
Digital vs Manual	94.7%	94.7%	(94.7%
NOVA View vs Manual	76.0%	86.3%	(72.7%
Digital vs NOVA View	69.6%	69.6%	69.6%

\*As percentage of samples that were positive with manual interpretation.

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

Not applicable

4. Clinical cut-off:

See analytical cut-off.

5. Expected values/Reference range:

The clinical validation study population included samples from 75 apparently healthy controls (different cohort from those used for cut-off LIU establishment) which were used to determine the reference range.

Out of the 75 samples tested at the Inova site, there were 4 (5.3%), 5 (6.7%) and 4 (5.3%) positive results with Manual, Digital, and NOVA View® classification respectively. With the NOVA View®, the average  $\pm$  SD was  $20 \pm 49$  LIU, with a median value of 9 LIU, and a range of 0-367 LIU, with non-normal distribution. The expected result in the normal population is negative; however, 10-20% of positivity may be seen in reference subjects according to published literature.

**N. Proposed Labeling:**

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

**O. Conclusion:**

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