NOVA Lite® DAPI ANA Kit

For *In Vitro* Diagnostic Use
CLIA Complexity: High

Inova Diagnostics
A Werfen Company

REF **704320** **Rx Only**

Intended Use

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.

Summary and Explanation of the test

The term "anti-nuclear antibodies" (ANA) describes a variety of autoantibodies that react with constituents of cell nuclei including DNA, RNA and several proteins and ribonucleoproteins [(1)]. These autoantibodies occur with high frequency in patients with systemic autoimmune rheumatic diseases (SARD), especially systemic lupus erythematosus (SLE) [(1-4)]. Indirect immunofluorescence (IIF) is the reference method for ANA testing [(5)]. Common substrates are thin sections of rodent organs or various types of cell lines. It is generally agreed that cell line substrates are preferable to organ sections since these rapidly dividing cells have higher levels of certain clinically relevant antigens, including centromere, SS-A (Ro), Scl-70 and PCNA/Cyclin. While ANA testing is an excellent screening test for SLE [(2, 6)] it is not a specific test. Patients with other connective tissue diseases such as Sjogren's syndrome (SS), mixed connective tissue disease (MCTD), rheumatoid arthritis (RA), scleroderma/systemic sclerosis (SSc), autoimmune liver diseases (AIL) and idiopathic inflammatory myopathy (IIM) are frequently positive, and low ANA titers may be observed in other disease states, such as infectious diseases, and even healthy people, especially in older population [(6)]. Up to 20% of serum samples from healthy individuals have been reported to have a positive ANA test [(7, 8)]. The substrate chosen for NOVA Lite DAPI ANA Kit is an optimally-fixed human epithelial (HEp-2) cell line, and the FITC (fluorescein 5-isothiocyanate) conjugate is Fc specific affinity purified anti-human IgG. These reagent parameters allow the NOVA Lite DAPI ANA kit to detect clinically relevant autoantibodies (including SS-A and Scl-70), which can remain undetected by some other commercial ANA tests. In addition, the IgG conjugate specificity eliminates physiologic false positive results due to normally occurring low titer IgM autoantibodies, often found in older, but otherwise healthy persons.

Principles of the Procedure

Samples are diluted to an initial titer of 1:80 in specimen diluent, incubated with the antigen substrate, and unbound antibodies are washed off. The substrate is then incubated with the anti-human IgG-FITC conjugate. The conjugate also contains a DNA-binding blue fluorescent dye, 4',6-diamidino-2-phenylindole (DAPI)[(9)] that is required for NOVA View use. The blue dye is not visible by traditional fluorescence microscope at the wavelength where FITC fluorescence is viewed. Unbound reagent is washed off, and the slides are coverslipped. Stained slides can be read and interpreted by traditional fluorescence microscopy, or can be scanned by NOVA View, followed by the review of the digital images by a trained operator. ANA positive samples will exhibit an apple green fluorescence corresponding to areas of the cell nuclei where autoantibody has bound. Some sera may contain antibodies reacting with cytoplasmic antigens, and will exhibit apple green fluorescence corresponding to areas of the cell cytoplasm where autoantibody has bound. When slides are assessed by NOVA View, digital images of representative areas of the well are captured. These digital images must be reviewed and interpreted from the computer monitor by a trained operator. When used with manual fluorescence microscopy, samples that are positive at 1:80 may be titered by performing a 2-fold serial dilution from the initial screening dilution with specimen diluent (i.e. 1:80, 1:160, 1:320, 1:640, 1:1280, 1:2560, etc.) to determine the endpoint titer.

Reagents

- HEp-2 (human epithelial cell) substrate slides; 12 wells/slide with desiccant.
- Anti-human IgG (Fc) FITC Conjugate with 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. Serves as sample diluent and wash buffer.
- Mounting Medium, containing 0.09% sodium azide
- Coverslips

Warnings

1. All human source material used in the preparation of kit controls for this product has been tested and found negative for antibody to HIV, HBsAg, and HCV by FDA cleared methods. No test method, however, can offer complete assurance that HIV, HBV, HCV or other infectious agents are absent. Therefore, the ANA Titratable Pattern and IFA System Negative Control should be handled with universal precautions, the same manner as potentially infectious material [(10)].
2. Sodium azide is used as a preservative in some kit components. Sodium azide is a poison and may be toxic if ingested or absorbed through the skin or eyes. Sodium azide may react with lead or copper plumbing to form potentially explosive metal azides. Flush sinks, if used for reagent disposal, with large volumes of water to prevent azide build-up.
3. Use appropriate personal protective equipment while working with the reagents provided.
4. Spilled reagents should be cleaned up immediately. Observe all federal, state and local environmental regulations when disposing of wastes.

Precautions

1. Substitution of components other than those provided in this system may lead to inconsistent results.
2. Incomplete or inefficient washing of IIF wells may cause high background.
3. Adaptation of slide processing for use with automated sample processors and other liquid handling devices, in whole or in part, may yield differences in test results from those obtained using the manual procedure. It is the responsibility of each laboratory to validate that their automated procedure yields test results within acceptable limits.
4. A variety of factors influence the assay performance. These include the temperature of the reagents, the strength of the microscope light source, the accuracy and reproducibility of the pipetting technique, the thoroughness of washing and the length of the incubation times during the assay. Careful attention to consistency is required to obtain accurate and reproducible results.
5. Strict adherence to the protocol is recommended.

Storage Conditions

1. Store all the kit reagents at 2-8°C. Do not freeze. Reagents are stable until the expiration date when stored and handled as directed.
2. 1X PBS II buffer is stable for 4 weeks at 2-8°C.

Specimen Collection

- This procedure should be performed with a serum specimen. Addition of azide or other preservatives to the test samples may adversely affect the results.
- Microbially contaminated, heat-treated samples or specimens containing visible particulates should not be used. Samples containing up to 10 mg/dL bilirubin, 200 mg/dL hemoglobin, 1000 mg/dL triglycerides, 224 mg/dL cholesterol, or 56 IU IgM rheumatoid factor did not show interference with the results generated with the NOVA Lite DAPI ANA Kit.
- Following collection, the serum should be separated from the clot. The following storage conditions are recommended for samples:

    1. Serum can be stored at room temperature for up to 24 hours.
    2. Serum can be stored at 2-8°C for up to 7 days.
    3. If the assay will not be completed within 7 days, or for shipment of the serum, freeze at -20°C or lower. Samples may be frozen and thawed up to 3 times. Frozen samples must be mixed well after thawing and prior to testing.

Procedure

Materials Provided

Item provided	Quantity
ANA HEp-2, QR Slide	20 x 12 well
FITC IgG Conjugate with DAPI	1 x 15 mL
ANA Titratable Pattern	1 x 0.5 mL
IFA System Negative Control	1 x 0.5 mL
PBS II Concentrate (40x)	2 x 25 mL
Mounting Medium	1 x 7 mL
Coverslips	1 x 20

Additional Materials Required But Not Provided

- Micropipettes to deliver 10-1000µL volume
- Distilled or deionized water
- Squeeze bottles or Pasteur pipets
- Moist chamber
- 1L containers (for diluting PBS)
- Coplin jar(s)
- Manual fluorescence microscope and/or NOVA View Automated Fluorescence Microscope

Method

Before you start

1. Bring all reagents and samples to room temperature (20-26°C) and mix well.
2. Dilute PBS II (40X) Concentrate: Dilute the PBS II (40X) Concentrate 1:40 by adding the contents of the PBS Concentrate bottle to 975mL of distilled or deionized water and mix thoroughly. The 1X PBS II buffer is used for diluting patient samples and as wash buffer.
3. Dilute Patient Samples: For initial screening, dilute patient samples 1:80 with 1X PBS II buffer (for example, add 10 µL of serum to 790 µL of 1XPBS II buffer), and mix well.

Assay procedure

1. Prepare Substrate Slides: Allow the substrate slide to reach room temperature prior to removal from its pouch. Label slides with pencil (if desired), and place it in a suitable moist chamber. Add 1 drop of the undiluted positive and the negative control to wells 1 and 2 respectively. Add 30 ± 10 µL of diluted patient samples to the remaining wells.

2. Slide Incubation: Incubate the slide for 30 ± 5 minutes in a moist chamber (a dampened paper towel placed flat on the bottom of a closed plastic or glass container will maintain the proper humidity conditions). Do not allow the substrate to dry out during the assay procedure.
3. Wash Slides: After incubation, use a plastic squeeze bottle or pipet to gently rinse off the serum with diluted PBS II buffer. Orient the slide and stream of PBS buffer so as to minimize wash-over and cross-contamination of samples between wells. Avoid directing the stream directly onto the wells to prevent substrate damage. Place the slides in a Coplin jar of 1XPBS II buffer for at least 5 minutes.
4. Addition of Fluorescent Conjugate: Remove slides (one at a time) from Coplin jar. Tap off the excess PBS buffer. Place the slide back in the moist chamber and immediately cover each well with a drop of FITC IgG Conjugate with DAPI. Incubate the slides for 30 ± 5 minutes.
5. Wash Slides: Repeat Step 3.
6. Coverslip: Coverslip procedures vary from lab to lab; however, the following procedure is recommended when slides are processed manually:
    a. Place a coverslip on a paper towel.
    b. Apply mounting medium in a continuous line to the bottom edge of the coverslip.
    c. Tap off the excess PBS buffer and touch the lower edge of the slide to the edge of the coverslip. Gently lower the slide onto the coverslip in such a way that the mounting medium flows to the top edge of the slide without air bubble formation or entrapment.

Quality Control

- The ANA Titratable Pattern and the IFA System Negative Control should be included in every run to ensure that all reagents and procedures perform properly. Additional suitable control sera may be prepared by aliquoting pooled human serum specimens and storing them at < -20°C. The test results are considered valid if all of the criteria listed below are met. If any of these criteria are not met, the test results are considered invalid, and the assay should be repeated.
    1. The undiluted ANA Titratable Pattern must produce a positive ANA reaction with homogeneous pattern ≥ 3+ intensity.
    2. The IFA System Negative Control must produce a negative ANA reaction.

- The ANA Titratable Pattern is suitable for endpoint titration. Once the endpoint titer is determined by the laboratory, the consistent analytical sensitivity of the assay can be checked by regular monitoring of the endpoint of the ANA Titratable Pattern. Every laboratory should determine the endpoint titer by using its own equipment, processes and microscope. The titers obtained may differ from laboratory to laboratory, depending on the microscope, light source, and magnification used to determine endpoint.

Interpretation of Results

Slides are examined and results are interpreted with a fluorescence microscope. It is recommended to use a 40x objective for final interpretation.

Alternatively, slides are placed on NOVA View, and analyzed by the instrument. The resulting digital images must be viewed and interpreted on the computer monitor by a trained operator. Refer to NOVA View Operator's Manual on how to read and interpret slides with NOVA View.

NOVA View identifies five characteristic patterns: homogeneous, speckled, centromere, nucleolar and nuclear dots. As IIF patterns can be mixed, non-characteristic, and variable, the software may not be able to identify a pattern for each positive sample. In these cases it reports the pattern as "Unrecognized". The final pattern in these cases is determined by the operator.

- **Negative Reaction:** A sample is considered negative if no specific staining of the nuclei and cytoplasm is present. Samples can exhibit various degrees of background staining due to heterophile antibodies or low-level non-specific autoantibodies to cytoplasmic constituents such as contractile proteins.
- **Positive Reaction:** A sample is considered positive if a specific staining of the nuclei is present..
- **Reactivity grade:** It is recommended to determine the fluorescence grade or intensity using these criteria:

    4+ Brilliant apple green fluorescence
    3+ Bright apple green fluorescence
    2+ Clearly distinguishable positive fluorescence
    1+ Lowest specific fluorescence that enables the nuclear and/or cytoplasmic staining to be clearly differentiated from the background fluorescence.

- **Pattern Interpretation:**

    A variety of patterns of nuclear and/or cytoplasmic staining can be exhibited depending on the types and relative amounts of autoantibodies present in the sample.
    The following types of staining patterns can be most frequently observed:

Homogeneous: A solid staining of the nucleus with or without apparent masking of the nucleoli, with positive solid staining of metaphase chromatin in mitotic cells.
Antibody specificities: most frequently antibodies against dsDNA, ssDNA, chromatin, histones.
Disease association: Suggestive of SLE, or other connective tissue diseases.

Speckled: A grainy appearing staining of the nucleus, generally without fluorescent staining in the nucleoli and without the staining of metaphase chromatin of mitotic cells.
Antibody specificities: most frequently antibodies against Sm, RNP, SS-A (Ro), SS-B (La). Anti-Scl-70 antibody usually produces a fine, diffuse speckled staining, often with additional nucleolar staining, but with staining of the metaphase chromatin of mitotic cells.
Disease association: Suggestive of SLE, or other connective tissue diseases.

Nucleolar: Large coarse speckles within the nucleus, generally less than 6 in number per cell, with or without occasional fine speckles and metaphase staining of the mitotic cells.
Antibody specificities: most frequently antibodies against fibrillarin, RNA polymerase, PM/Scl**,** Th/To and other nucleolar antigens.

Disease association: High titers are prevalent in progressive systemic sclerosis (scleroderma).
Centromere: A discrete speckled staining pattern. The nuclear speckles are very discrete and are usually present in some multiple of 46, with condensed speckled staining of metaphase chromatin of mitotic cells.
Antibody specificities: antibodies against Centromere Protein A or B (CENP-A, CENP-B).
Disease association: Highly suggestive of CREST syndrome, a variant of progressive systemic sclerosis. CREST is a form of systemic sclerosis with prominent calcinosis (C), Raynaud's phenomenon (R), esophageal dysmotility (E), sclerodactyly (S), and telanglectasias (T).
Nuclear dots: Few discrete small speckles in the nucleus with negative metaphase mitotic cells. The number of discrete speckles may vary (3-20 per cell) depending on the antigen specificity.
Antibody specificities: antibodies against Sp-100, PML, p80-coilin.
Disease association: Highly suggestive of primary biliary cirrhosis.
Both in the presence and absence of nuclear staining, cytoplasmic staining can be present due to antibodies directing against cytoplasmic structures, such as mitochondria, ribosomal protein P, histidyl tRNA synthetase (Jo-1), Golgi apparatus and others.

It is important to caution the user about relying on patterns to determine autoantibody specificity, except for the centromere pattern, which is very well defined and the pattern is characteristic.

Refer to the NOVA View Operator's Manual for guidance regarding interpretation of the results from the digital images generated by NOVA View.

Limitations of the Procedure

1. High-titered ANA is suggestive of connective tissue disease but should not be considered diagnostic. The ANA result should be considered in combination with other serological results as well as the overall clinical history including signs and symptoms of the patient.
2. ANA patterns often change as the sample is titered out to endpoint. This phenomenon is due to lower titer antibodies dropping below the sensitivity of the system as more dilute sample is tested.
3. A variety of external factors influence the test sensitivity including the type of fluorescence microscope, the strength of the light source, and the magnification used.
4. It is recommended that pencil be used to label the slides. Use of any other writing material may cause artifactual staining.
5. All Coplin jars used for slide washing should be free from all dye residues. Use of Coplin jars containing dye residue may cause artifactual, non-specific staining.
6. Results of this assay should be used in conjunction with clinical findings and other serological tests.
7. The assay performance characteristics have not been established for matrices other than serum.
8. NOVA View should only be used with patient samples diluted at 1:80.
9. Single well titer determinations by NOVA View are estimates only and should be validated by manual titration using traditional manual fluorescence microscopy only.
10. NOVA View is only for use with reagents that are indicated for use with the device.
11. All software-aided results must be confirmed by the trained operator.
12. NOVA View is for use by a trained operator in a clinical laboratory setting.

Precision

Altogether 43 samples, representing negative and positive reactions, all five patterns (homogeneous, speckled, nucleolar, centromere, nuclear dots), and fluorescence grade levels from 0 to 4+ were tested in one or two runs per day, in triplicates or in duplicates for five days, in three separate experiments. All slides were read with NOVA View, and digital images were reviewed by a trained operator. Results were expressed in fluorescence grades (0 to 4+). Moreover, for 30 samples out of the 43, the same slides were also read with manual fluorescence microscope.
All replicates for each sample were within ± one fluorescence grade from each other within each run (repeatability) and between runs and days (reproducibility), with digital image interpretation and manual interpretation.

Reproducibility

A cohort of 120 selected serum samples were tested with the NOVA Lite DAPI ANA kit at Inova, and at two external clinical sites. The samples were selected so as to include all the characteristic ANA patterns, and various fluorescence intensity levels, from low positive to high positive. At each site, the slides were assessed by NOVA View, and the generated digital images were interpreted by the same operator who then read the same slides with traditional fluorescence microscope. Between-sites 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. Agreement was also calculated between methods at each site. Results are shown below:

Manual reading	**Site#1 vs Site #2**	**Site#1 vs Site #3**	**Site#2 vs Site #3**
Average Neg. Agreement % (95% CI)	97.6 (94.3-100.0)	99.2 (97.4-100.0)	96.8 (93.1-99.3)
Average Pos. Agreement % (95% CI)	97.4 (94.0-100.0)	99.1 (97.0-100.0)	96.6 (92.7-99.2)
Overall agreement % (95% CI)	97.5 (92.9-99.5)	99.2 (95.4-100.0)	96.87(91.7-99.1)

NOVA View reading	**Site#1 vs Site #2**	**Site#1 vs Site #3**	**Site#2 vs Site #3**
Average Neg. Agreement % (95% CI)	100 (100.0-100.0)	96.6 (93.3-99.3)	96.6 (93.3-99.3)
Average Pos. Agreement % (95% CI)	100 (100.0-100.0)	96.4 (92.4-99.2)	96.4 (92.4-99.2)
Overall agreement % (95% CI)	100 (97.0-100.0)	96.7 (91.7-99.1)	96.7 (91.7-99.1)

Digital image reading	Site#1 vs Site #2	Site#1 vs Site #3	Site#2 vs Site #3
Average Neg. Agreement % (95% CI)	95.9 (91.7-99.2)	95.4 (91.2-98.6)	92.9 (87.7-97.1)
Average Pos. Agreement % (95% CI)	95.8 (91.6-99.2)	94.5 (89.6-98.3)	92.0 (86.2-96.7)
Overall agreement % (95% CI)	95.8 (90.5-98.6)	95.0 (89.4-98.1)	92.5 (86.2-96.5)

Total agreement between methods % (95% CI)	Site#1	Site#2	Site#3
Manual vs digital image reading	99.2 (95.4-100.0)	95.8 (90.5-98.6)	96.7 (91.7-99.1)
Manual reading vs NOVA View	99.2 (95.4-100.0)	96.7 (91.7-99.1)	96.7 (91.7-99.1)
Digital image reading vs NOVA View	100 (97.0-100.0)	95.8 (90.5-98.6)	98.3 (94.1-99.8)

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). Summary table of pattern agreement is shown below.

Reproducibility cohort n=120	Number (%) of samples with pattern agreement*		
	Site#1	**Site#2**	**Site#3**
Manual vs Digital	55 (96.5%)	57 (95.0%)	54 (96.4%)
Manual vs NOVA View	45 (78.9%)	50 (83.3%)	45 (80.4%)
Digital vs NOVA View	44 (77.2%)	48 (80.0%)	45 (80.4%)

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

Fluorescence intensity grades as determined by manual reading and digital image reading were within ± one dilution step from each other in between-sites comparison as shown below:

Percent samples with grades within ± one dilution step from each other	Site#1 vs Site #2	Site#1 vs Site #3	Site#2 vs Site #3
Manual reading	99.2	93.3	95.0
Digital image reading	96.6	87.5	91.7

It is noted that pattern interpretation and fluorescence grade differences includes variability that is related to the experience and training of the operator.

Expected Values

The expected result in the normal population is negative. However, a variable proportion of the healthy population has been described to have positive ANA test. [6-8] Seventy-five (75) serum samples from apparently healthy blood donors were tested with the NOVA Lite DAPI ANA Kit. Out of the 75 samples, there were 4 (5.3%), 5 (6.7%) and 4 (5.3%) positive results with manual reading, digital reading, and according to NOVA View classification, with the highest fluorescence grade being 2+.

Clinical Performance

In a clinical validation study, 463 samples have been tested to assess the clinical performance of the assay. The composition of the cohort and the number of positive tests are shown in the table below:

		Number of positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual reading**	**Digital reading**
Healthy control	75	4	4	5
HBV (Hepatitis B positive)	20	1	5	3
HCV(Hepatitis C positive)	5	2	0	2
HIV(HIV positive)	5	2	0	2
Syphilis	5	3	0	3
SLE (Systemic Lupus Erythematosus)	75	60	54	60
SSc (Systemic Sclerosis)	20	19	19	19
SS (Sjogren’s syndrome)	20	12	9	13
AIL (Autoimmune Liver Disease)	20	20	16	20
RA (Rheumatoid Arthritis)	20	15	11	14
MCTD (Mixed Connective Tissue Disease)	21	10	10	10
Idiopathic inflammatory myopathy (IIM)	26	6	7	6
Fibromyalgia	25	6	9	6
Anti-MPO/anti-PR3 positive	26	1	3	4
Crohn's/Inflammatory bowel disease	20	8	9	8
Autoimmune thyroiditis/Anti-TPO positive	24	2	4	3

		Number of positive samples		
Sample type	**Number of samples**	**NOVA View**	**Manual reading**	**Digital reading**
Celiac disease/anti-tTG positive	24	3	4	3
Drug induced lupus (DIL)	25	4	5	5
Other	7	1	2	1
Total	**463**	**179**	**171**	**187**

Sensitivity was calculated on SLE separately, and on the combination of the systemic autoimmune rheumatic diseases (SARD) (SLE + SSc + SS + MCTD + IIM + DIL) plus autoimmune liver disease (AIL) population. Specificity was calculated on the total control population excluding healthy subjects, according to NOVA View interpretation, digital image reading results and manual (microscopic) reading results. The control population includes samples from patients with RA.

Site #1:

	Sensitivity % (95% CI)		**Specificity %**
	SLE	**SARD+AIL**	**(95% CI)**
	(N=75)	**(N=186)**	**(N=174)**
NOVA View	80.0 (69.2-88.4)	69.4 (62.2-75.9)	75.3 (68.2-81.5)
Manual reading	72.0 (60.4-81.8)	62.9 (55.5-69.9)	74.1 (67.0-80.5)
Digital reading	80.0 (69.2-88.4)	69.9 (62.8-76.4)	72.4 (65.1-78.9)

The same 463 samples were also tested at two external clinical sites. The results are shown below:

Site #2:

	Sensitivity % (95% CI)		**Specificity %**
	SLE	**SARD+AIL**	**(95% CI)**
	(N=75)	**(N=186)**	**(N=174)**
NOVA View	72.0 (60.4-81.8)	62.9 (55.5-69.9)	77.0 (70.0-83.0)
Manual reading	70.7 (59.0-80.6)	65.6 (58.3-72.4)	67.2 (59.7-74.2)
Digital reading	73.3 (61.9-82.9)	62.98 (55.5-69.9)	75.3 (68.2-81.5)

Site #3:

	Sensitivity % (95% CI)		**Specificity %**
	SLE	**SARD+AIL**	**(95% CI)**
	(N=75)	**(N=186)**	**(N=174)**
NOVA View	82.7 (72.2-90.4)	72.0 (65.0-78.4)	69.0 (61.5-75.7)
Manual reading	82.7 (72.2-90.4)	71.0 (63.9-77.4)	67.2 (59.7-74.2)
Digital reading	81.3 (70.7-89.4)	69.4 (62.2-75.9)	71.3 (63.9-77.9)

The overall agreement between manual reading, digital image reading and NOVA View results at the three testing sites is shown below:

Total agreement between methods % (N=463)	**Site #1**	**Site #2**	**Site #3**
Manual reading vs NOVA View	89.6 (86.5-92.3)	89.8 (86.7-92.4)	87.0 (83.6-90.0)
Manual reading vs digital reading	91.4 (88.4-93.8)	92.2 (89.4-94.5)	92.2 (89.4-94.5)
Digital reading vs NOVA View	97.0 (95.0-98.3)	96.3 (94.2-97.8)	92.2 (89.4-94.5)

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). Summary table of pattern agreement is shown below.

Clinical cohort n=463	**Number (%) of samples with pattern agreement***		
	Site #1	**Site #2**	**Site #3**
Manual vs Digital	162 (94.7%)	174 (91.6%)	200 (95.7%)
Manual vs NOVA View	130 (76.0%)	164 (86.3%)	152 (72.7%)
Digital vs NOVA View	119 (69.6%)	168 (88.4%)	157 (75.1%)

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

Fluorescence intensity grades as determined by digital image reading were within ± one dilution step from those of determined by traditional manual reading in 96.3%, 99.1% and 99.6% of the samples at the three sites.

The Reference Sera for Antinuclear Antibodies from the Center of Disease Control and Prevention (CDC)[11, 12] have also been tested with the NOVA Lite DAPI ANA Kit. All reference sera produced the expected ANA pattern. Results are shown in the table below:

CDC ANA Human Reference Serum ID	Expected ANA pattern	Known antibody specificity	Fluorescent pattern, manual reading	Fluorescent pattern, digital image reading
Reference Serum #1	Homogeneous/ Rim	nDNA	Homogeneous	Homogeneous
Reference Serum #2	Speckled	SS-B/La	Speckled	Speckled
Reference Serum #3	Speckled	RNP, SS-B/La, SS-A/Ro	Speckled	Speckled
Reference Serum #4	Speckled	U1-RNP	Speckled	Speckled
Reference Serum #5	Speckled	Sm	Speckled	Speckled
Reference Serum #6	Nucleolar	Fibrillarin	Nucleolar	Nucleolar
Reference Serum #7	N/A	SS-A/Ro	Speckled	Speckled
Reference Serum #8	Centromere	Centromere	Centromere	Centromere
Reference Serum #9	N/A	Scl-70	Homogeneous	Homogeneous
Reference Serum #10	N/A	Jo-1	ANA negative; Cytoplasmic speckled	ANA negative; Cytoplasmic speckled
Reference Serum #11	N/A	PM-Scl	Nucleolar	Nucleolar
Reference Serum #12	N/A	Ribosomal P	Negative*	Negative*

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

Cross-reactivity

Cross reactivity was examined on 114 samples that were part of the clinical validation study. Samples were from patients with autoimmune thyroid disease/thyroid-peroxidase (TPO) antibodies, celiac disease/anti-tissue transglutaminase (tTG) antibodies, anti-myeloperoxidase (MPO) and anti-proteinase 3 (PR3) antibodies, patients with Crohn's disease/inflammatory bowel disease and rheumatoid arthritis.

The number and distribution of the population is shown in the Table below, together with the ANA positivity rate. Considering all 114 samples, the observed positivity rate was 25% for NOVA View results, 27% for digital image reading, and 28% for manual reading. The positivity rate was 15% for NOVA View results, 19% for digital image reading, and 21% for manual reading, when rheumatoid arthritis samples were not included. This positivity rate is in line with the expected results and the published literature.[5-8] ANA positivity in RA has previously been described with high frequency. RA-33 antibodies are present in up to 36% of RA patients, and anti-histone antibodies have also been identified in the sera of RA subjects. [13-16]

Cross-reactivity		**Positivity rate**					
		NOVA View		**Manual reading**		**Digital reading**	
Sample type	**Number**	**Number**	**%**	**Number**	**%**	**Number**	**%**
Anti-MPO/anti-PR3	26	1	4%	3	12%	4	15%
Crohn's/Inflammatory bowel disease	20	8	40%	9	45%	8	40%
Autoimmune thyroiditis	24	2	8%	4	17%	3	13%
Celiac disease	24	3	13%	4	17%	3	13%
Rheumatoid arthritis	20	15	75%	11	55%	14	70%
Total	**114**	**29**	**25%**	**31**	**28%**	**32**	**27%**

Agreement between NOVA Lite DAPI and the predicate device

Results that were obtained with the NOVA Lite DAPI ANA kit, using 1:80 serum dilution, were compared to those obtained with the predicate device that uses 1:40 serum dilution, and anti-human IgG conjugate without DAPI.

The comparison study was performed on 410 samples: 400 clinically characterized sera, and 10 samples with known ANA patterns. All slides were interpreted with traditional fluorescence microscopy. 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:

	Number of samples	**Number of positive at 1:40**	**Number of positive at 1:80**
Apparently healthy controls	150	41	17
SLE (Systemic Lupus Erythematosus)	100	85	81
SS (Sjogren's syndrome)	30	23	21
SSc (Systemic Sclerosis)	30	20	15
Idiopathic inflammatory myopathy (IIM)	10	9	7
MCTD (Mixed Connective Tissue Disease)	20	12	12

	Number of samples	Number of positive at 1:40	Number of positive at 1:80
Infectious disease	30	6	4
RA (Rheumatoid arthritis)	30	20	17
Centromere antibody positive	5	5	5
Mitochondrial antibody positive	5	4	5
Total	410	224	184

Agreement between the two methods is shown below:

	Positive Agreement % (95% CI)	Negative Agreement % (95% CI)	Total Agreement % (95% CI)
1:80 vs 1:40 dilution	79.9 (74.1-85.0)	97.3 (91.5-100.0)	87.7 (84.2-90.8)

179 samples were positive according to both methods. The number of pattern discrepancies (not including negative/positive discrepancies, but including patterns interpreted as “other”) was five (2.2% of samples that tested positive in 1:40 dilution).

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

Fluorescence grade in 1:80 dilution	Fluorescence grade in 1:40 dilution					
	0	**1+**	**2+**	**3+**	**4+**	**Total**
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*

Out of the 410 samples, 45 samples were positive in 1:40 dilution that were negative in 1:80. Moreover, four samples that were negative in 1:40 dilution were positive in 1:80 dilution. Out of the 45 samples, 28 were in from the healthy + infectious diseases population, and 3 had the diagnosis of RA. Two samples were from patients with IIM, two from patients with Sjogren’s syndrome, five had SLE, and five had systemic sclerosis. All these samples had a fluorescence intensity grade of 1+, except for one myositis sample that had a grade of 2+.

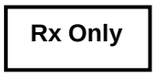
The prevalence of ANA in the healthy population (n=150) was 27.3% when sera were tested in 1:40 dilution, and was 11.3% when sera were tested in 1:80 dilution.

Overall clinical sensitivity and specificity is shown below:

	Sensitivity % (95% CI)		Specificity* % (95% CI) (N=60)
	SLE (N=100)	**SARD (N=190)**	
1:40 dilution	85.0 (76.5-91.4)	78.4 (71.9-84.0)	56.7 (43.2-69.4)
1:80 dilution	81.0 (71.9-88.2)	71.6 (64.4-77.9)	65.0 (51.6-76.9)

SARD: Systemic Autoimmune Rheumatic Disease (includes SLE, SSc, SS, MCTD and IIM)
**Control samples include RA and infectious disease population*

Symbols Used
Símbolos utilizados

	In Vitro diagnostic medical device Producto sanitario para diagnóstico in vitro
	European Conformity Conformidad europea
	European Authorized Representative Representante europeo autorizado
	Prescription Only per US FDA Solo con receta, conforme a la FDA de EE. UU.
	Temperature Limitation Límites de temperatura
	Batch Code Código de lote
	Catalogue or part number Número de catálogo o componente
	Manufacturer Fabricante
	Use by Caducidad
	Contains Sufficient Contenido suficiente para
	Consult instructions for use Consulte las instrucciones de uso

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