510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY

I Background Information:

A 510(k) Number

K203599

B Applicant

Immuno Concepts N.A., Ltd.

C Proprietary and Established Names

Image Navigator by Immuno Concepts, Immuno Concepts IgG Anti-nDNA Fluorescent Test System

D Regulatory Information

Product Code(s)	Classification	Regulation Section	Panel
KTL	Class II	21 CFR 866.5100 - Antinuclear Antibody Immunological Test System	IM - Immunology
PIV	Class II	21 CFR 866.4750 - Automated Indirect Immunofluorescence Microscope And Software-Assisted System	IM - Immunology

II Submission/Device Overview:

A Purpose for Submission:

Migration of previously cleared assay to a previously cleared instrument

B Measurand:

Anti-double stranded DNA (dsDNA) IgG autoantibodies

C Type of Test:

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

III Intended Use/Indications for Use:

A Intended Use(s):

The Immuno Concepts IgG Anti-nDNA Fluorescent Test System is for in vitro diagnostic use for the qualitative detection and semiquantitation of anti-nDNA antibodies of the IgG class in human serum by manual fluorescent microscopy or with the Image Navigator Fluorescence Semiautomated Microscope. The Immuno Concepts IgG Anti-nDNA Fluorescent Test System is to be used as an aid in the diagnosis of Systemic Lupus Erythematosus (SLE) in conjunction with other clinical and laboratory findings. A trained operator must confirm results generated with the Image Navigator semi-automated device and software.

B Indication(s) for Use:

Same as Intended Use

C Special Conditions for Use Statement(s):

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

D Special Instrument Requirements:

For use with the Image Navigator by Immuno Concepts (K160265)

IV Device/System Characteristics:

A Device Description:

The IgG Anti-nDNA Fluorescent Test System is an in vitro diagnostic (IVD) device for use either in a conventional (i.e., human read with a microscope) immunofluorescent assay or by analysis with the Image Navigator. The same kit, with no modifications, is used for both conventional and Image Navigator analysis.

Assay kit components:

IgG anti-nDNA Fluorescent Antibody Test System Device Description		
Kit Component	Part Number	Description
Reactive reagents		
Substrate Slides	3007: 7-well *C. luciliae* slides	Multi-well fixed monolayer of *Crithidia luciliae* on glass slides
	3014: 14-well *C. luciliae* slides	
nDNA positive control	3021	1.0 mL positive control human serum with IgG antibodies specific to nDNA antigens
Titratable control serum	3026	0.5 mL positive control human serum to be treated as an undiluted patient sample; titer ranges provided by lot
Negative control	3031	1.0 mL human control serum

IgG anti-nDNA Fluorescent Antibody Test System Device Description		
Kit Component	Part Number	Description
Fluorescent antibody reagent (conjugate)	3009G: 9.0 mL 3075G: 23 mL	goat-anti-human IgG conjugated to FITC
Non-reactive components		
PBS buffer powder	1011	phosphate-buffered saline powder (0.01M, pH 7.4±0.2), for 1L reconstituted buffer
Mounting medium	1111	5.0 mL semi-permanent glycerol-based mounting medium
Coverslips	1042	Ten 24×64 mm No. 1 glass coverslips

Instrument:

The Immuno Concepts IgG Anti-nDNA Fluorescent Test System can be used by manual fluorescent microscopy equipped with 495 nm exciter filter and 515 nm barrier filter or with the Image Navigator fluorescence semi-automated microscope. Image Navigator by Immuno Concepts digital microscopy instrument and software were cleared in K160265.

B Principle of Operation:

The Immuno Concepts IgG anti-nDNA Test System uses the indirect fluorescent antibody technique, also referred to as the *Crithidia luciliae* Immunofluorescence Test (CLIFT). Patient samples are incubated on substrate slides with *C. luciliae*. If antibodies reactive to specific protozoan antigens are present in the sample, a stable antigen-antibody complex is formed. After washing to remove non-specific and unbound antibodies, the sample is incubated with an anti-human antibody conjugated to fluorescein.

If autoantibodies are present, autoantibodies bound to the substrate can be visualized with the aid of a fluorescent microscope, with a staining pattern characteristic of the protozoan kinetoplast. Smooth or peripheral staining of the kinetoplast with a bright fluorescence located near the flagellar region of the *C. luciliae* is considered positive, representative of autoantibodies against dsDNA. No fluorescence or any other pattern specificities (i.e., nuclear, basal body, flagellar) are considered negative; concurrent staining of the kinetoplast alongside these other patterns is considered positive.

Qualitative "screening" of samples is done at an initial dilution of 1:10. A sample is negative if the kinetoplast fluorescence at 1:10 is less than or equal to the negative control well. A sample is positive if the kinetoplast shows discernible staining with fluorescence greater than the negative control well at 1:10 titer. The 1:10 dilution will be reflex tested to endpoint titration for semi-quantitation. Doubling dilutions (e.g., 1:20, 1:40) of the initial dilution are made and tested with *C. luciliae* substrate as above. The final dilution that retains kinetoplast fluorescent staining is the semi-quantitative endpoint titer (ET). Fluorescence intensity (FI) from 0 to +4 may also be reported by following guidelines for fluorescent antibody reagents established by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia:

4+ Brilliant yellow-green (maximal fluorescence)
3+ Less brilliant yellow-green fluorescence
2+ Definite cell pattern but dim fluorescence
1+ Very subdued fluorescence
0 No fluorescence

V Substantial Equivalence Information:

A Predicate Device Name(s):

Image Navigator by Immuno Concepts, Igg Anti-ndna Fluorescent Test System; Model # 3040g

B Predicate 510(k) Number(s):

K160265, K013432

C Comparison with Predicate(s):

Device & Predicate Devices:	**K203599**	**K013432**
Device Trade Name	IgG Anti-nDNA Fluorescent Test System	Immuno Concepts IgG Anti-nDNA Fluorescent Test System
Intended Use/ Indications For Use	This test system is for in vitro diagnostic use for the qualitative detection and semi-quantitation of anti-nDNA antibodies of the IgG class in human serum by manual fluorescent microscopy or with the Image Navigator Fluorescence Semiautomated Microscope. This test system is to be used as an aid in the diagnosis of systemic lupus erythematosus in conjunction with other clinical and laboratory findings. A trained operator must confirm results generated with the Image Navigator semiautomated device and software.	This is an indirect fluorescent antibody test for the semi-quantitative detection of IgG anti-nDNA antibodies in human serum. This test system is to be used as an aid in the diagnosis of systemic lupus erythematosus.
Methodology	Indirect immunofluorescence (IFA)	Same
Sample Matrix	Serum	Same
Conjugate	Goat anti-human IgG FITC	Same
Storage	2-10°C	Same
Target indication(s)	Systemic Lupus Erythematosus (SLE)	Same
Antigen Substrate	*Crithidia luciliae*	Same
Starting Dilution	1:10	Same
Assay Cut-off	1:10	Same
Results	Qualitative, semi-quantitative (titer, fluorescence intensity)	Same
Image Acquisition and Analysis	Manual (Mode A) or Image Navigator semi-automated (Mode B)	Manual (Mode A) conventional fluoromicroscopy

Image Evaluation	Operator-dependent	Operator-Dependent
Software	Image Navigator does not provide recommended qualitative outcome (i.e. positive or negative) to the user following image acquisition	n/a

VI Standards/Guidance Documents Referenced:

Org	Std ID	Version	Date	Title
CLSI	EP05	A3	Sep 2014	Evaluation of Precision of Quantitative Measurement Procedures
CLSI	EP07	Ed. 3	Dec 2002	Interference Testing in Clinical Chemistry
CLSI	EP12	A2	Jan 2014	User Protocol for Evaluation of Qualitative Test Performance
CLSI	EP28	A3c	Oct 2010	Defining, Establishing, and Verifying Reference Intervals in the Clinical Laboratory

VII Performance Characteristics (if/when applicable):

A Analytical Performance:

1. Precision/Reproducibility:

To assess the repeatability of the assay on the semi-automated monitor mode (Mode B), two negative sample and 10 nDNA-positive patient serum samples representing low, medium, and high titers were evaluated. Using the semi-automated monitor Image Navigator reading mode, two users per site, at three sites read each sample in triplicate for 10 runs over five days, two runs per day, for a total of 180 datapoints per sample.

The qualitative results (i.e., positive or negative kinetoplast fluorescence) across all sites and readers for these 14 samples matched 1675/1680 total calls for a total overall agreement of 99.7% with 95% Confidence Interval (CI) of 99.3−99.9%. Agreement of each sample across all users (*n*=6) and cross all sites are summarized in the table below:

Total Agreement Semi-Automated/Monitor Image Navigator Reading Mode − across all sites

Sample ID	Expected Titer	*n*	Total Agreement, across all sites		
			Qualitative	±1 titer	±1 FI
1	(−)	180	100% (97.5−100%)	*n/a*	*n/a*
2	(−)	180	100% (97.5−100%)	*n/a*	*n/a*
3	20	180	97.2% (93.5−99.0%)	93.3% (88.6−96.2%)	96.7% (92.8−98.6%)
4	40	180	100% (97.5−100%)	100% (97.5−100%)	100% (97.5−100%)
5	160	180	100% (97.5−100%)	99.4% (96.6−99.9%)	97.2% (93.5−99.0%)
6	320	180	100% (97.5−100%)	99.4% (96.6−99.9%)	100% (97.5−100%)
7	640	180	100% (97.5−100%)	98.9% (95.8−100%)	99.4% (96.6−99.9%)
8	640	180	100% (97.5−100%)	97.8% (94.2−99.3%)	98.9% (95.8−100%)
9	640	180	100% (97.5−100%)	94.4% (90.0−97.1%)	99.4% (96.6−99.9%)
10	640	180	100% (97.5−100%)	98.3% (95.0−99.7%)	98.3% (95.0−99.7%)
11	1280	180	100% (97.5−100%)	97.8% (94.2−99.3%)	100% (97.5−100%)
12	2560	180	100% (97.5−100%)	98.3% (95.0−99.7%)	98.9% (95.8−100%)
13	2560	180	100% (97.5−100%)	97.8% (94.2−99.3%)	98.9% (95.8−100%)
14	2560	180	100% (97.5−100%)	95.0% (90.6−97.5%)	97.8% (94.2−99.3%)

Multi-site reproducibility agreement measures using the semi-automated monitor mode for qualitative results (i.e., positive or negative kinetoplast fluorescence) across all sites and readers for these 14 samples matched 1675/1680 total calls for a total overall agreement of 99.7% (95% CI: 99.3−99.9%). Agreement measures for endpoint titer between-users and between-sites are presented in the tables below:

Reproducibility: Semi-automated monitor mode – % Titer Agreement (±1 titer), with 95% CI

		Site 1		Site 2		Site 3	
		User 1	User 2	User 1	User 2	User 1	User 2
Site 1	User 1		94.2% (92.2–95.7%)	97.4% (95.9–98.3%)	97.4% (95.9–98.3%)	97.5% (96.1–98.4%)	96.8% (95.2–97.9%)
	User 2			96.3% (94.6–97.4%)	96.3% (94.6–97.4%)	96.4% (94.7–97.5%)	95.7% (93.9–97.0%)
Site 2	User 1				99.4% (98.5–99.8%)	99.6% (98.7–99.9%)	98.9% (97.8–99.5%)
	User 2					99.6% (98.7–99.9%)	98.9% (97.8–99.5%)
Site 3	User 1						99.0% (98.0–99.6%)
	User 2						

In addition, multi-site and multi-reader components were incorporated the clinical method comparison in §VII.B-C below, as pre-specified in the special controls in 21 CFR §866.4750.

Lot-to-lot reproducibility was evaluated in K013432.

2. Linearity:

To assess endpoint titration, four anti-nDNA positive samples, representing multiple endpoint titer levels were evaluated. The sample was analyzed by two readers using semi-automated monitor reading. Consistent positivity was found throughout dilution to endpoint and a consistent titer endpoint was found between readers and methods (within ±1 titer). Results from this study are summarized in the following table where fluorescence intensity (FI) results are reported at each dilution of individual samples for each of two representative readers, for each representative duplicate:

Linearity, Mode A semi-automated monitor method

Sample ID	Expected Titer	Reader	Replicate	Observed Titer (Dilution^{-1})								
				10	20	40	80	160	320	640	1280	2560
1	>2560	1	1	*nd*[1]				+4	+4	+4	+4	+3
1	>2560	1	2	*nd*[1]				+4	+4	+4	+4	+3
1	>2560	2	1	*nd*[1]				+4	+4	+4	+4	+3
1	>2560	2	2	*nd*[1]				+4	+4	+4	+4	+3
2	>2560	1	1	*nd*[1]						+3	+2	+1
2	>2560	1	2	*nd*[1]						+3	+2	+1
2	>2560	2	1	*nd*[1]						+2	+1	+1
2	>2560	2	2	*nd*[1]						+2	+1	+1
3	1:160	1	1	*nd*[1]		+2	+2	+1	0	*nd*[2]		
3	1:160	1	2	*nd*[1]		+1	+1	0	0	*nd*[2]		
3	1:160	2	1	*nd*[1]		+3	+2	+1	0	*nd*[2]		
3	1:160	2	2	*nd*[1]		+3	+2	+1	0	*nd*[2]		

Linearity, Mode A semi-automated monitor method												
Sample ID	Expected Titer	Reader	Replicate	Observed Titer (Dilution^{-1})								
				10	20	40	80	160	320	640	1280	2560
4	1:20	1	1	*nd*[1]	+1	0	0	*nd*[2]				
4	1:20	1	2	*nd*[1]	+1	0	*nd*[2]					
4	1:20	2	1	*nd*[1]	+3	+2	0	*nd*[2]				
4	1:20	2	2	*nd*[1]	+3	0	*nd*[2]					
5	1:20	1	1	+1	0	0	0	*nd*[2]				
5	1:20	1	2	+1	0	0	0	*nd*[2]				
5	1:20	2	1	+3	+2	+1	0	*nd*[2]				
5	1:20	2	2	+3	+3	+2	0	*nd*[2]				
6	(−)	1	1	0	*nd*[2]							
6	(−)	1	2	0	*nd*[2]							
6	(−)	2	1	0	*nd*[2]							
6	(−)	2	2	0	*nd*[2]							
[1] not done, presumptive positive staining [2] not done, presumptive negative staining												

Results were acceptable.

3. Analytical Specificity/Interference:

Interference

Analytical interference studies were performed following CLSI EP07, 3rd Ed. Four specimens (one negative, low, medium, and high-titer positives) were tested in triplicate by one reader for three concentrations of each of five endogenous interferents and 10 exogenous interferents. Interferents were spiked by predilution and addition of 10% (v/v) of final total sample volume, while controls were diluted with addition of 10% (v/v) diluent. (Rheumatoid factor or control serum) were diluted to two relevant testing concentrations at [25 and 50% (v/v)].

No interference was detected with conjugated bilirubin up to 40 mg/dL, hemoglobin up to 1.0 g/dL, triglycerides up to 1.5 g/dL, cholesterol up to 400 mg/dL, and rheumatoid factor IgM up to 60 IU/mL.

No interference was detected with methylprednisolone (representative corticosteroid) to 0.78 mg/dL, ibuprofen to 21.9 mg/dL, naproxen to 36 mg/dL, hydroxychloroquine to 3.3 mg/dL, cyclophosphamide to 54.9 mg/dL, mycophenolate mofetil to 4.2 mg/dL, methotrexate to 136 mg/dL, azathioprine to 0.258 mg/dL, rituximab to 7.6 mg/mL, and atorvastatin to 75 µg/dL.

Expected Values

The performance of reference materials was assessed by testing standards from the U.S. Centers for Disease Control (CDC),[1] as well as the Association of Medical Laboratory Immunologists (AMLI). These materials were tested by three readers at each of three sites, a different instrument at each site. Results from testing these materials is described in the table below:

[1] Chan EK, Fritzler MJ, Wiik A, Andrade LE, Reeves WH, Tincani A, Meroni PL; IUIS/WHO/AF/CDC Committee for the Standardization of Autoantibodies in Rheumatic and Related Diseases. "AutoAbSC.Org -- Autoantibody Standardization Committee in 2006" *Autoimmun Rev*, 2007 Sep; 6(8):577-80. doi: 10.1016/j.autrev.2007.05.001. PMID: 17854752.

Performance of ANA Reference Materials, semi-automated monitor method

ANA Reference Material	Expected		Reader/Site		
	Specificity	Titer	1	2	3
SLR AMLI-9 dsDNA	dsDNA	(+)		1:80	
CDC IS2072: ANA Reference Serum 1	homogenous/ dsDNA	1:160–1280	1:320	1:320	1:80
CDC IS2073: ANA Reference Serum 2	SS-B/La	(−)	(−)	(−)	(−)
CDC IS2074: ANA Reference Serum 3	speckled	(−)	(−)	(−)	(−)
CDC IS2075: ANA Reference Serum 4	U1 RNP	(−)	(−)	(−)	(−)
CDC IS2076: ANA Reference Serum 5	Sm	(−)	(−)	(−)	(−)
CDC IS2100: ANA Reference Serum 6	U3 RNP	(−)	(−)	(−)	(−)
CDC IS2105: ANA Reference Serum 7	SS-A/Ro	(−)	(−)	(−)	(−)
CDC IS2134: ANA Reference Serum 8	centromere	(−)	(−)	(−)	(−)
CDC IS2135: ANA Reference Serum 9	Scl-70	(−)	(−)	(−)	(−)

4. Assay Reportable Range:

   The maximum endpoint titer assessed in analytical validation studies was to 1:2560.

5. Traceability, Stability:

   Traceability

   A recognized standard or reference material for anti-dsDNA antibodies for immunofluorescence were not used; see Expected Values above.

   Stability

   Reagent stability studies were reviewed in K013432.

6. Detection Limit:

   Not applicable.

7. Assay Cut-Off:

   Clinical performance studies evaluated a 1:10 initial dilution cutoff, by convention. Detectable fluorescence at this 1:10 initial dilution is considered qualitatively positive.

B Comparison Studies:

Smooth or peripheral staining of the kinetoplast with a bright fluorescence located near the flagellar region of the *C. luciliae* is considered positive, representative of autoantibodies against double-stranded DNA (dsDNA). No fluorescence or any other pattern specificities (i.e., nuclear, basal body, flagellar) are considered negative; concurrent staining of the kinetoplast alongside these other patterns is considered positive. Qualitative “screening” of samples is done at an initial dilution of 1:10. A sample is negative if the kinetoplast fluorescence at 1:10 is less than or

equal to the negative control well. A sample is positive if the kinetoplast shows discernible staining with fluorescence greater than the negative control well at 1:10 titer.

The 1:10 dilution will be reflex tested to endpoint titration for semi-quantitation. Doubling dilutions (e.g., 1:20, 1:40) of the initial dilution are made and tested with *C. luciliae* substrate as above. The final dilution that retains kinetoplast fluorescent staining is the semi-quantitative endpoint titer (ET). Fluorescence intensity (FI) from 0 to +4 may also be reported by following guidelines for fluorescent antibody reagents established by the Centers for Disease Control and Prevention (CDC), Atlanta, Georgia:

4+ Brilliant yellow-green (maximal fluorescence)
3+ Less brilliant yellow-green fluorescence
2+ Definite cell pattern but dim fluorescence
1+ Very subdued fluorescence
0 No fluorescence

1. Method Comparison with Predicate Device:

Four hundred and seventy-nine clinical samples (see Section VII.C below) were read by three operators per site, at three sites, in both reading modes (Mode A manual/conventional or Mode B semi-automated/monitor) for a total of 8,622 datapoints. Qualitatively positive samples at the 1:10 initial dilution were titered to extinction for endpoint titer values.

The table below summarizes the qualitative agreement of Mode B versus Mode A, for all sites based on ≥2/3 consensus:

		Method Comparison: Summary		
		Mode A: Manual		
		(+)	(−)	
Mode B: Semi-auto/ Montior	(+)	55	0	55
	(−)	0	424	424
		55	424	479

	Agreement	(95% CI)
Positive Percent Agreement (PPA):	100%	(92.2–100%)
Negative Percent Agreement (NPA):	100%	(98.2–100%)

The table below summarizes the comparison of Mode B semi-automated monitor Image Navigator reading to Mode A conventional manual reading, across all three sites, based on ≥2/3 consensus for the endpoint titer:

nDNA Clinical Method Comparison: – (+/*n*) Multi-Site Titer Agreement

		Mode A: conventional manual predicate							
		(−)	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Mode B: semi-automated monitor	(−)	not done		0/1	0/6	0/8	0/3	0/15	0/7
	1:10			0/1	0/6	0/8	0/3	0/15	0/7
	1:20			1/1	0/6	0/8	0/3	0/15	0/7
	1:40			0/1	5/6	0/8	0/3	0/15	0/7
	1:80			0/1	1/6	7/8	0/3	0/15	0/7
	1:160			0/1	0/6	1/8	2/3	3/15	0/7

nDNA Clinical Method Comparison: – (+/*n*) Multi-Site Titer Agreement

		Mode A: conventional manual predicate							
		(−)	1:10	1:20	1:40	1:80	1:160	1:320	1:640
	1:320			0/1	0/6	0/8	1/3	12/15	0/7
	1:640			0/1	0/6	0/8	0/3	0/15	7/7
	total *n*	0	0	1	6	8	3	15	7

The tables below summarize the comparison of Mode B semi-automated monitor Image Navigator endpoint titers to Mode A conventional manual predicate reads, for individual sites, based on ≥2/3 consensus among readers at each site for the endpoint titer:

nDNA Clinical Method Comparison Site 1: – (+/*n*) Titer Agreement

		Mode A: conventional manual predicate							
		(−)	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Mode B: semi-automated monitor	(−)	n/a	0/1	0/2	0/10	0/7	0/6	0/8	0/6
	1:10		0/1	1/2	0/10	0/7	0/6	0/8	0/6
	1:20		1/1	1/2	0/10	0/7	0/6	0/8	0/6
	1:40		0/1	0/2	9/10	1/7	0/6	0/8	0/6
	1:80		0/1	0/2	1/10	6/7	1/6	0/8	0/6
	1:160		0/1	0/2	0/10	0/7	5/6	7/8	1/6
	1:320		0/1	0/2	0/10	0/7	0/6	1/8	1/6
	1:640		0/1	0/2	0/10	0/7	0/6	0/8	4/6
	total *n*	0	1	2	10	7	6	8	6

nDNA Clinical Method Comparison Site 2: – (+/*n*) Titer Agreement

		Mode A: conventional manual predicate							
		(−)	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Mode B: semi-automated monitor	(−)	n/a		0/1	0/5	0/2	0/7	0/12	0/13
	1:10			0/1	0/5	0/2	0/7	0/12	0/13
	1:20			1/1	0/5	0/2	0/7	0/12	0/13
	1:40			0/1	5/5	0/2	0/7	0/12	0/13
	1:80			0/1	0/5	2/2	0/7	0/12	0/13
	1:160			0/1	0/5	0/2	7/7	0/12	0/13
	1:320			0/1	0/5	0/2	0/7	12/12	0/13
	1:640			0/1	0/5	0/2	0/7	0/12	13/13
	total *n*	0	0	1	5	2	7	12	13

nDNA Clinical Method Comparison Site 3: – (+/*n*) Titer Agreement

		Mode A: conventional manual							
		(−)	1:10	1:20	1:40	1:80	1:160	1:320	1:640
Mode B: semi-automated monitor	(−)	n/a		0/1	0/5	0/2	0/7	0/12	0/13
	1:10			0/1	0/5	0/2	0/7	0/12	0/13
	1:20			1/1	0/5	0/2	0/7	0/12	0/13
	1:40			0/1	5/5	0/2	0/7	0/12	0/13
	1:80			0/1	0/5	2/2	0/7	0/12	0/13
	1:160			0/1	0/5	0/2	7/7	0/12	0/13
	1:320			0/1	0/5	0/2	0/7	12/12	0/13
	1:640			0/1	0/5	0/2	0/7	0/12	13/13
	total *n*	0	0	1	5	2	7	12	13

The table below summarizes agreement of qualitative results from this study by individual laboratory sites, and the semi-quantitative endpoint titer and fluorescence intensity parameters, comparing Mode B semi-automated monitor reading to Mode A conventional manual reading, based on total number of 8,622 reads for 3 readers × 3 sites:

Method Comparison: Mode B (monitor) v Mode A (manual), by read with 95% CI

Parameter	*n*	PPA		NPA	
Qualitative (+)/(−)	503/3808	99.8%	(98.8−100%)	99.8%	(98.8−100%)
Endpoint Titer (±1)	492	86.8%	(83.5−89.5%)		

2. Matrix Comparison:

Not applicable; serum is the only claimed and validated matrix.

C Clinical Studies:

1. Clinical Sensitivity:

For clinical sensitivity, 172 serum samples from patients with systemic lupus erythematosus (SLE) were tested by 3 operators/site × 3 sites × 2 reading modes (see §VII.B.1 above). These samples represented patients who met diagnostic criteria published by the American College of Rheumatology (ACR) in 1982 and updated in 1997 (*n*=143),[2] or jointly by ACR and the European League Against Rheumatism (EULAR) in 2019 (*n*=29).[3]

The table below summarizes the qualitative sensitivity of Mode B semi-automated monitor reading, against the ground truth SLE condition, for individual sites based on ≥2/3 consensus among readers at each site:

Clinical Sensitivity Per Site, with 95% CI

Reading Mode	Site	*n*		Sensitivity	
		total	(+)	point estimate	(95% CI)
Mode B: Semi-auto/Monitor	1	172	52	30.2%	(23.8−37.5%)
	2	172	52	30.2%	(23.8−37.5%)
	3	172	52	30.2%	(23.8−37.5%)

[2] Hochberg MC. "Updating the American College of Rheumatology revised criteria for the classification of systemic lupus erythematosus" *Arthritis Rheum*, 1997 Sep; 40(9):1725. doi: 10.1002/art.1780400928. PMID: 9324032.

[3] Aringer M, Costenbader K, Daikh D, Brinks R, Mosca M, Ramsey-Goldman R, Smolen JS, Wofsy D, Boumpas DT, Kamen DL, Jayne D, Cervera R, Costedoat-Chalumeau N, Diamond B, Gladman DD, Hahn B, Hiepe F, Jacobsen S, Khanna D, Lerstrøm K, Massarotti E, McCune J, Ruiz-Irastorza G, Sanchez-Guerrero J, Schneider M, Urowitz M, Bertsias G, Hoyer BF, Leuchten N, Tani C, Tedeschi SK, Touma Z, Schmajuk G, Anic B, Assan F, Chan TM, Clarke AE, Crow MK, Czirják L, Doria A, Graninger W, Halda-Kiss B, Hasni S, Izmirly PM, Jung M, Kumánovics G, Mariette X, Padjen I, Pego-Reigosa JM, Romero-Diaz J, Rúa-Figueroa Fernández Í, Seror R, Stummvoll GH, Tanaka Y, Tektonidou MG, Vasconcelos C, Vital EM, Wallace DJ, Yavuz S, Meroni PL, Fritzler MJ, Naden R, Dörner T, Johnson SR. "2019 European League Against Rheumatism/American College of Rheumatology Classification Criteria for Systemic Lupus Erythematosus" *Arthritis Rheumatol*, 2019 Sep; 71(9):1400-1412. doi: 10.1002/art.40930. PMID: 31385462.

2. Clinical Specificity:

For clinical specificity, 307 serum samples representing 16 diagnoses and diagnostic categories, as listed below, were tested by 3 operators/site × 3 sites × 2 reading modes (see §VII.B.1 above). The table below summarizes the qualitative specificity of Mode B semi-automated monitor reading, for individual diagnoses or diagnostic categories based on ≥2/3 consensus among the three sites:

Clinical Specificity Per Diagnostic Category, with 95% CI

Disease	*n*	(+)	Specificity (95% CI)
ANCA-associated vasculitis (AAV)	30	1	96.7% (83.3–99.4%)
Antiphospholipid syndrome (APS)	30	0	100% (86.5–100%)
Autoimmune hepatitis (AIH)	10	0	100% (67.9–100%)
Primary biliary cholangitis (PBC)	10	0	100% (67.9–100%)
Primary sclerosing cholangitis (PSC)	10	0	100% (67.9–100%)
Polymyositis/dermatomyositis (PM/DM)	30	0	100% (86.5–100%)
Progressive systemic sclerosis (PSSc)	30	0	100% (86.5–100%)
Rheumatoid arthritis (RA)	30	0	100% (86.5–100%)
Sjögren's syndrome (SjS)	30	0	100% (86.5–100%)
Fibromyalgia	10	0	100% (67.9–100%)
Mixed connective tissue disease (MCTD)	15	2	86.7% (60.9-97.5%)
Undifferentiated connective tissue disease (UCTD)	15	0	100% (76.1–100%)
Epstein-Barr virus (EBV)	7	0	100% (59.6–100%)
Hepatitis B virus (HBV)	10	0	100% (67.9–100%)
Hepatitis C virus (HCV)	10	0	100% (67.9–100%)
Lymphoma	30	0	100% (86.5–100%)

3. Other Clinical Supportive Data:

Combining the results from the clinical sensitivity and specificity cohorts above, the confusion matrix below summarizes clinical performance across clinical sites based on ≥2/3 consensus using the Mode B semi-automated monitor reading mode:

Clinical Performance Summary, with 95% CI

		SLE		
		(+)	(−)	
Immuno Concepts IgG Anti- nDNA	(+)	52	3	55
	(−)	120	304	424
		172	307	479

Sensitivity:	30.2%	(23.8–37.5%)
Specificity:	99.0%	(97.0–99.8%)
PPV:	94.6%	(84.6–98.7%)
NPV:	71.7%	(67.2–75.8%)

The clinical method comparison studies were performed with multi-site reproducibility parameters, testing the cohort of 479 patient samples by 3 operators/site × 3 sites × 2 reading

modes, in addition to the precision studies above in §VII.B.1. The table below summarizes the comparison between sites (i.e. site-to-site) of Mode B semi-automated monitor Image Navigator reading, based on ≥2/3 consensus among readers at each site for each parameter, qualitative (+/−), endpoint titer, and fluorescence intensity (FI):

Clinical Method Comparison: Between-Site Reproducibility

Sites:	1 v 2	1 v 3	2 v 3
(+)	40/40	40/40	40/41
(−)	424/424	423/424	423/423
±1 Titer	21/40	33/40	37/41
±1 FI	463/464	463/464	456/464

The table below summarizes the comparison within each site between individual operators, of Mode B semi-automated monitor Image Navigator reading, for each parameter, qualitative (+/−), endpoint titer, and fluorescence intensity:

Clinical Method Comparison: Within-Site Inter-Operator Reproducibility

Site:	1			2			3		
Operator:	1 v 2	1 v 3	2 v 3	1 v 2	1 v 3	2 v 3	1 v 2	1 v 3	2 v 3
(+)	40/42	40/42	40/42	40/40	40/40	40/40	41/45	41/45	41/43
(−)	415/422	420/422	415/422	424/424	424/424	424/424	417/419	417/419	419/421
±1 Titer	40/41	37/41	40/41	40/40	40/40	40/40	41/42	38/42	40/42
±1 FI	456/464	461/464	457/464	464/464	464/464	464/464	458/464	458/464	460/464

D Clinical Cut-Off:

Refer to assay cut-off.

E Expected Values/Reference Range:

Samples from 120 apparently healthy subjects were tested by 2 operators/site × 3 sites × 3 days × 2 reading modes (see §VII.B.1 above). 100% of all reads (*n*=4320) were negative.

VIII Proposed Labeling:

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

IX Conclusion:

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