510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K172745

B. Purpose for Submission:

New device

C. Measurand:

IgG Anti-Nuclear Antibodies (ANA)

D. Type of Test:

Qualitative or semi-quantitative, indirect immunofluorescence

E.Applicant:

IMMCO Diagnostics, Inc.

F.Proprietary and Established Names:

ImmuGlo HEp-2 Elite IFA

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

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use:

   The ImmuGlo HEp-2 Elite IFA is an indirect immunofluorescence antibody test for the qualitative or semi-quantitative detection of anti-nuclear antibodies (ANA) of the IgG isotype in human serum utilizing standard HEp-2 cells and engineered HEp-2 cells as a substrate. The ImmuGlo HEp-2 Elite IFA is intended for use as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.

2. Indication for use:

   Same as Intended Use

3. Special conditions for use statement:

   For Prescription Use Only

4. Special instrument requirements:

   Fluorescence microscope for manual operation
   200× or higher magnification, 490 nm excitation filter, 510 nm barrier filter

I. Device Description:

The kit is an indirect immunofluorescence assay (IFA) to detect IgG anti-nuclear antibodies (ANA) against proteins presented in HEp-2 cell lines. The standard test kit contains:

- Substrate slides: barcoded glass slides with standard and engineered DFS70-knock-out HEp-2 cells attached in a monolayer in the slide wells
- ANA homogeneous pattern positive control
- ANA DFS70 pattern positive control
- Negative Control
- Conjugate: anti-human IgG FITC conjugate containing Evan's blue counterstain. Binds fluorescein to reactive antibodies reacting against HEp-2 antigens
- Buffered diluent provided for specimen dilutions
- Wash buffer: phosphate buffered saline (PBS) diluent removes reagents and unbound antibodies after incubation steps
- Mounting medium provided for coverslipping of slides to view under fluorescence microscope
- Coverslips

Optional components:

The standard kit format supplies the Conjugate and Evan’s blue counterstain as one pre-formulated reagent, however, they may also be supplied separately to accommodate laboratory workflow. The formulation is intended to be separate but functionally identical.

J. Substantial Equivalence Information:

1. Predicate device name:

   ImmuGlo ANTINUCLEAR ANTIBODY (ANA) TEST (HEP-2) CELLS

2. Predicate 510(k) number:

   K883883

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Intended Use	The ImmuGlo HEp-2 Elite IFA is an indirect immunofluorescence antibody test for the qualitative or semi-quantitative detection of anti-nuclear antibodies (ANA) of the IgG isotype in human serum utilizing standard HEp-2 cells and engineered HEp-2 cells as a substrate. The ImmuGlo HEp-2 Elite IFA is intended for use as an aid in the diagnosis of systemic rheumatic diseases in conjunction with other clinical and laboratory findings.	Indirect immunofluorescence (IF) antibody test for the detection and quantitation of anti-nuclear antibodies (ANA) in human serum
Methodology	Indirect immunofluorescence	Same
Matrix	Serum	Same
Detection of antibodies	Anti-nuclear antibodies	Same
Component Set	Includes positive control, negative control, conjugate, diluent, wash buffer, mounting medium, substrate slides, coverslips	Same
Conjugate	FITC IgG	Same
Screening dilution	1:40	Same
Reading	Fluorescence microscope	Same
Storage	2–8° C	Same

Differences		
Item	Device	Predicate
Substrate	Standard HEp-2 cells mixed with engineered HEp-2 with the *PSIP1* gene knocked out in 1:9 ratio	Standard HEp-2 cells
Positive control	ANA homogenous, DFS70 Ab	ANA homogenous

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

Org	Standard ID	Version	Date	Title
CLSI	EP05	A3	Sep 2014	Evaluation of Precision Performance of Quantitative Measurement Methods
CLSI	EP06	A	Apr 2003	Evaluation of the Linearity of Quantitative Measurement Procedures
CLSI	EP07	A2	Dec 2002	Interference Testing in Clinical Chemistry
CLSI	EP12	A2	Jan 2014	User Protocol for Evaluation of Qualitative Test Performance

L. Test Principle:

In the indirect IFA method used in this kit, patient sera are incubated on HEp-2 cell substrates to allow binding of antibodies. Any antibodies not bound are removed by washing with buffer. Bound antibodies of the IgG class are detected by incubation of the substrate with fluorescein-labeled anti-human IgG conjugate. Reactions are observed under a fluorescence microscope equipped with appropriate filters. The presence of nuclear and cytoplasmic patterns AC-1 to AC-28 as described by ICAP[1] is demonstrated by green fluorescence of specific structures in the cells. A majority (~90%) of the cells present on the substrate are devoid of a functional *PSIP1* gene which eliminates the relevant antigenic target of the DFS70 pattern. This may aid in discrimination of ANA homogeneous and speckled patterns from the DFS pattern.

It is recommended that patient samples demonstrating specific fluorescence reactions at a dilution of 1:40 be reported as positive. Each laboratory must determine its own normal values to account for differences in microscopy systems, personnel, and training. The titers are determined by testing serial dilutions of patient samples and reported as the highest reciprocal dilution with a positive reaction.

M. Performance Characteristics:

1. Analytical performance:

   All results presented below met the manufacturer's pre-determined acceptance criteria.

   Interpretation of Results: Results should be reported as negative, positive, or, if end-point titer has been determined, positive with titer. It is recommended that patient samples demonstrating specific fluorescence reactions at a dilution of 1:40 be reported as positive. Each laboratory must determine its own normal values to account for differences in microscopy systems, personnel, and training. Users should only read only fields that contain specific staining of the HEp-2 substrates.

[1]. Chan EK, Damoiseaux J, Carballo OG, Conrad K, de Melo Cruvinel W, Francescantonio PL, Fritzler MJ, Garcia-De La Torre I, Herold M, Mimori T, Satoh M, von Mühlen CA, Andrade LE. "Report of the First International Consensus on Standardized Nomenclature of Antinuclear Antibody HEp-2 Cell Patterns 2014-2015" *Front Immunol*, 2015; 6:412. doi: 10.3389/fimmu.2015.00412. PMID: 2634773

The International Consensus on Antinuclear Antibody Patterns report recommends a standardized nomenclature and reporting format for IFA patterns.[1]. Major nuclear patterns that are recommended to be reported include Homogeneous (AC-1), Speckled (AC-2, AC-4, AC-5), Centromere (AC-3), Discrete Nuclear Dots (AC-6, AC-7) and Nucleolar (AC-8, AC-9, AC-10). Major cytoplasmic patterns include Fibrillar (AC-15, AC-16, AC-17), Speckled (AC-18, AC-19, AC-20), Reticular/AMA (AC-21), Polar Golgi-like (AC-22) and Rods and rings (AC-23). Including the most commonly and mandated to be reported patterns described above, a total of 28 patterns are described in the ICAP reference.[1]

Interpretation of DFS70 pattern on HEp-2 ELITE IFA substrate: In the ~10% baseline HEp-2 cells in each well, a dense fine speckled pattern will be observed on interphase nuclei. Chromatin-associated staining is seen on mitotic nuclei. The remaining ~90% of HEp-2 cells have the *PSIP1* gene encoding the LEDGF antigen knocked out and therefore will not produce a similar pattern. If 10% of the HEp-2 cells have brighter fluorescence corresponding to DFS70 pattern and rest of the cells present additional patterns or fine speckled signal above cut-off, it indicates the presence of mixed patterns. Closer analysis of such patterns is essential to confirm if other antibody specificity is present in addition to the DFS70 pattern associated with LEDGF/*PSIP1*. Due to the high prevalence of AC-2 patterns in an ANA screening population and relatively low association with autoimmune rheumatic diseases, the ICAP committee recommends all clinical labs report the DFS70 pattern.

a. Precision/Reproducibility:

The sponsor modeled precision studies based on CLSI guideline EP05-A3.

***Repeatability*:**

To assess repeatability of the ImmuGlo HEp-2 Elite IFA, 8 negative and 17 positive samples representing various patterns and positivities were tested: six replicates × two runs/day × five days × two operators = 120 replicate measures for each sample. There was ≥ 90% agreement for pattern calls for all samples. One speckled sample was reported with 93% pattern agreement.

***Reproducibility*:**

To assess variability, 10 positive samples with various patterns and positivities and one negative sample were tested in triplicate in 10 runs (two runs/day × five days) at three different sites, resulting in 90 datapoints for each sample for each outcome . Global agreement values for pattern identity and titer (±1 dilution value) are provided in the table below:

Reproducibility of HEp-2 Elite IFA substrate across all three sites				
Sample	Pattern	Endpoint Titer	Pattern Agreement	Titer Agreement (±1 dilution)
1	DFS70	80	100%	100%
2*	Cytoplasmic	80	100%	100%
	Nuclear dots	80	100%	100%
3	Speckled	80	100%	100%
4	Centromere	80	100%	100%
5	Speckled	80	100%	100%
6	Homogeneous	40	93%	100%
7	DFS70	80	100%	100%
8	Homogeneous	160	100%	93%
9*	Cytoplasmic	1280	100%	100%
	Nuclear dots	1280	100%	100%
10	Nucleolar	1280	100%	100%
11	Negative	0	100%	100%

* samples 2 and 9 are mixed pattern specificity, primary pattern is listed first

Within-Site Reproducibility: There was ≥ 90% agreement for pattern call and titer agreement (±1 dilution) for all samples at all sites. All results showed 100% agreement for all patterns and titers at sites 2 and 3. A site four samples showed 90% agreement; all remaining samples showed 100% agreement. These samples had homogeneous patterns.

Between-Site Reproducibility: To examine the site-to-site variability of pattern calls and endpoint titer values, the reproducibility datasets were reorganized to compare each site in a pairwise fashion. Results are provided in the table below:

Reproducibility: Pattern and titer agreement for HEp-2 Elite IFA substrate between sites; for Titer Agreement values, a range of ±1 dilution was the acceptance criterion							
Sample	Pattern	Site 1 v Site 2		Site 1 v Site 3		Site 2 v Site 3	
		Pattern Agreement	Titer Agreement	Pattern Agreement	Titer Agreement	Pattern Agreement	Titer Agreement
1	DFS70	100%	100%	100%	100%	100%	100%
2*	Cytoplasmic	100%	100%	100%	100%	100%	100%
	Nuclear dots	100%	100%	100%	100%	100%	100%
3	Speckled	100%	100%	100%	100%	100%	100%
4	Centromere	100%	100%	100%	100%	100%	100%
5	Speckled	100%	100%	100%	100%	100%	100%
6	Homogeneous	100%	100%	90%	100%	90%	100%
7	DFS70	100%	100%	100%	100%	100%	100%
8	Homogeneous	100%	90%	100%	90%	100%	100%
9*	Cytoplasmic	100%	100%	100%	100%	100%	100%
	Nuclear dots	100%	100%	100%	100%	100%	100%
10	Nucleolar	100%	100%	100%	100%	100%	100%
11	Negative	100%	100%	100%	100%	100%	100%

* samples 2 and 9 are mixed pattern specificity, primary pattern is listed first

Lot-to-Lot Reproducibility: A lot-to-lot reproducibility study was performed using three reagent lots. Samples were selected to test negative to strong positive specimens representing all major patterns, including DFS70. Results of the study are presented in the table below:

Lot-to-lot Reproducibility of Qualitative Agreement			
	Lot 1 vs. Lot 2	Lot 1 vs. Lot 3	Lot 2 vs. Lot 3
Positive % Agreement (95% CI)	100% (99.4–99.9%)	100% (99.4–99.9%)	100% (99.4–99.9%)
Negative %Agreement (95% CI)	99% (98.7–99.7%)	99% (98.7–99.7%)	99% (98.7–99.7%)
Overall % Agreement (95% CI)	100% (99.3–99.8%)	100% (99.3–99.8%)	100% (99.3–99.8%)

Pattern identities were compared between lots. The number of positive specimens with consensus patterns for each lot are provided below. As determined by consensus of results, the specimens represented homogeneous, speckled, DFS70, centromere, nucleolar, cytoplasmic, and negative. Both definitive patterns and negative results were considered as pattern agreement. The results are presented in the table below:

Lot-to-lot Reproducibility of Pattern Agreement		
Lot	n	% Pattern Agreement
1	1497	99.6%
2	1496	99.6%
3	1498	99.6%
1-2-3	4491	99.6%

b. Linearity/assay reportable range:

To assess linearity of the ImmuGlo HEp-2 Elite IFA, 16 strong positive samples with various patterns were diluted from 1:40 to end-point. Two samples were tested from each of the main ANA patterns, including mixed-pattern samples.

The following terminology is used to describe fluorescence intensity results:

Fluorescence intensity interpretation	
Intensity	**Interpretation**
0	Negative. No fluorescence signal above background, or specific pattern
1+	Weak fluorescence and a distinguishable pattern
2+	Clearly distinguishable pattern with higher fluorescence signal relative to 1+ category
3+	Clearly distinguishable pattern with higher fluorescence signal relative to 2+ category
4+	Clearly distinguishable pattern with higher fluorescence signal relative to 3+ category

The samples showed decreasing fluorescence intensity with increased dilution depicted in table below. Patterns did not change when the samples were diluted.

Fluorescence intensity as a function of increasing sample dilution on HEp-2 Elite IFA substrate										
Sample	**Pattern(s)**	**Dilution^{-1}**								
		40	**80**	**160**	**320**	**640**	**1280**	**2560**	**5120**	**10240+**
1	Speckled	4+	4+	3+	3+	2+	2+	1+	1+	0
2	Speckled	4+	4+	3+	3+	2+	1+	0	0	0
3	Homogenous	4+	4+	3+	2+	2+	1+	1+	1+	1+
4	Homogenous	3+	3+	2+	2+	2+	2+	1+	1+	1+
5	Dense Fine Speckled (DFS)	2+	2+	2+	2+	1+	1+	0	0	0
6	DFS	3+	3+	3+	3+	2+	2+	1+	1+	0
7	Centromere	3+	3+	2+	2+	1+	1+	0	0	0
8	Centromere	3+	3+	2+	1+	1+	1+	1+	0	0
9	Nucleolar	3+	3+	3+	2+	1+	1+	0	0	0
10	Nucleolar	3+	3+	2+	2+	1+	0	0	0	0
11	Anti-Mitochondrial (AMA)	4+	4+	4+	3+	3+	3+	3+	2+	2+
12	1st: AMA	4+	4+	3+	3+	3+	3+	2+	2+	1+
	2nd: Nuclear Dots (ND)	3+	3+	3+	3+	3+	3+	2+	1+	1+
13	1st: DFS	3+	3+	3+	3+	2+	2+	1+	1+	0
	2nd: ND	3+	3+	2+	1+	1+	1+	0	0	0
14	1st: DFS	3+	3+	2+	1+	1+	0	0	0	0
	2nd: ND	2+	2+	1+	1+	1+	0	0	0	0
15	Nuclear Rim	4+	4+	4+	4+	3+	3+	2+	2+	1+
16	Nuclear Rim	3+	2+	1+	1+	1+	0	0	0	0

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

Traceability: A recognized standard for anti-nuclear antibodies is not available.

Stability: The sponsor performed accelerated, real time, and open kit/reagent stability studies as part of design control to assign expiration dating to components and as part of ongoing quality control/quality assurance analysis. Accelerated and open kit studies were performed on three lots of components/reagents. Based on these studies, the sponsor established an 18-month expiration date. Accelerated stability studies supported maximum shelf life of 18 months from date of manufacture. Open kit stability studies supported a maximum opened shelf life of 18 months from date of opening.

d. *Detection limit:*

Not applicable

e. Analytical specificity:

Cross-reactivity: Potentially cross-reactive disease sera were tested for ANA antibodies using the ImmuGlo HEp-2 Elite IFA, including 254 samples from non-systemic autoimmune rheumatic disease autoimmune disease conditions (ANCA-associated vasculitis, celiac disease, Crohn's disease, pernicious anemia, primary biliary cholangitis, Raynaud's syndrome, rheumatoid arthritis, autoimmune thyroiditis, and ulcerative colitis), 13 inflammatory disease conditions (atopic dermatitis, psoriasis), 57 cancer samples (breast, colorectal, ovarian, pancreatic, prostate), and 173 infectious disease samples (CMV, HBV, HCV, HIV, HSV, Lyme disease, mononucleosis, Rubella, syphilis, and toxoplasmosis). Positive prevalence in these cohorts was as expected in literature and comparable to the predicate device. See section M.3, below for the results of these studies.

Interference: The sponsor modeled interference studies based on CLSI EP07-A2. Interference was studied by mixing sera with known ANA antibody levels with potentially interfering substances and evaluating the deviation from expected results. No significant interference was demonstrated for the following substances at the levels indicated: hemoglobin (2 g/L), unconjugated bilirubin (342 μmol/L), rheumatoid factor (100 U/mL), cholesterol (13 mmol/L), triglycerides (3.7 mmol/L), naproxen (2170 μmol/L), ibuprofen (2425 μmol/L), prednisone (0.84 μmol/L), mycophenylate mofetil (14.01 μg/mL), hydroxychloroquine (388 ng/mL), cyclophosphamide (1437 μmol/L), rituximab (1.143 mg/mL) and belimumab (0.939 mg/mL).

While results using suspect specimens will generally produce the correct qualitative result, testing of grossly hemolyzed or lipemic samples is not recommended as stated in the product insert.

f. Assay cut-off:

The assay cutoff of 1:40 was established from literature. The recommended screening dilution is 1:40, at which a result with fluorescence intensity 1+ – 4+ and a recognizeable pattern is reported as positive. The manufacturer suggests laboratories perform doubling dilutions to establish their own titering protocols. The titers of 1:40 and 1:80 are considered low titers, 1:160 and 1:320 are considered medium titers, and 1:640 and greater are considered high titers.

2. Comparison studies:

The sponsor modeled qualitative comparison studies based on CLSI EP12-A2.

a. Method comparison with predicate device:

The ImmuGlo HEp-2 Elite IFA was tested in comparison to the predicate HEp-2 substrate using sera from 587 patients, corresponding to the clinical performance

cohorts (see Section M.3 below). Positive and negative percent agreement values were calculated, with 95% confidence intervals and results are presented in the table below:

Qualitative (positive or negative) performance of HEp-2 Elite IFA versus predicate ANA IFA				
		Predicate		
		ANA(+)	**ANA(–)**	
HEp-2 Elite	**ANA(+)**	297	5	302
	ANA(–)	1	288	289
		298	293	591

Positive Percent Agreement: 99.7% (98.2% – 99.9%)
Negative Percent Agreement: 98.3% (96.1% – 99.3%)

Pattern identity in the table below depicts the comparison of patterns between theHEp-2 Elite IFA and the predicate ANA IFA.

Pattern agreement of HEp-2 Elite IFA versus predicate ANA IFA												
		Predicate HEp-2										
		Ho	Sp	Cyt	DFS	Cent	AMA	Nucl	Esoteric*	Mixed	Neg	**Total**
HEp-2 Elite	Homogeneous	77	0	0	0	0	0	0	0	0	0	77
	Speckled	1	122	0	0	0	0	0	0	0	0	123
	Cytoplasmic	0	0	19	0	0	0	0	0	0	0	19
	DFS	0	0	0	0	0	0	0	0	0	0	0
	Centromere	0	0	0	0	19	0	0	0	0	0	19
	AMA	0	0	0	0	0	14	0	0	0	0	14
	Nucleolar	0	0	0	0	0	0	26	0	0	1	27
	Exotics*	0	0	0	0	0	0	0	13	0	0	13
	Mixed	1	0	0	0	0	0	0	0	5	0	6
	Negative	2	2	0	0	0	0	1	0	0	288	293
	Total	81	124	19	0	19	14	27	13	5	289	591

Key
Ho: Homogeneous
Sp: Speckled
DFS: Dense fine speckled
Cent: Centromere
AMA: anti-mitochondrial antibodies
Nucl: Nucleolar
Esoteric*: sum of the following patterns – nuclear dots, nuclear rim, centrosome
Neg: Negative

To evalute performance in DFS-positive samples, a cohort of 122 DFS antibody-suspected samples was studied separately. This enriched cohort was tested on both the predicate HEp-2 device and the HEp-2 ELITE IFA system. The Sponsor stated that 32 of these samples were reported on the predicate to be difficult to positively distinguish DFS from homogeneous or speckled patterns and described as '*Homogeneous/Speckled/DFS unable to be discriminated*'.

Results are summarized below:

Pattern agreement for "DFS70 suspected" samples				
		Predicate		
		DFS(+)	**DFS(–)***	
HEp-2 Elite	**DFS70(+)**	89	32	121
	DFS70(–)	0	0	0
		89	32	121

*includes 'Homogeneous/Speckled/DFS unable to be discriminated'
PPA: 100% (94.8–100%)
NPA: 0% (0–13.3%)

To further evaluate the ability of the HEp-2 Elite IFA substrate to successfully determine the DFS pattern, a reader study was performed. A total of 243 samples were selected to represent ANA patterns and negative samples, with particular emphasis on DFS, as well as homogeneous and speckled, as discrimination of these two patterns is most confounded with the DFS pattern. Samples were randomized, then tested on both the HEp-2 Elite IFA and predicate, and each substrate read by three independent readers. All readers were blinded to the expected pattern and to each others' pattern calls.

Pattern identity calls were compared between the HEp-2 Elite IFA and the predicate device for each individual reader. In this analysis, a pattern call was considered agreement if the HEp-2 Elite IFA device reported the same pattern as the predicate. In addition, aHEp-2 Elite IFA to predicate comparison was made for consensus reads, in which at least any two-of-three readers agree on the identity of a pattern call. Results are presented in the table below:

Positive and negative pattern agreement on HEp-2 Elite IFA substrate, compared to predicate for individual readers (Readers 1–3), and for consensus read of 2/3 readers. *n* values depict samples positive or negative by the predicate device.

Pattern		HEp-2 Elite IFA v Predicate ANA IFA							
		Reader 1		Reader 2		Reader 3		Consensus (≥2/3 Readers)	
		n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
All*	PPA	176	90.9% (85.7–94.3%)	170	81.2% (74.6–86.3%)	207	90.8% (86.1–94.0%)	177	87.0% (81.3–91.2%)
	NPA	85	77.6% (67.7–85.2%)	107	62.6% (53.2–71.2%)	65	55.4% (43.3–66.8%)	92	71.7% (61.8–79.9%)
DFS70**	PPA	26	100% (87.1–100%)	21	95.2% (77.3–99.2%)	42	100% (91.6–100%)	26	100% (87.1–100%)
	NPA	217	94.0% (90.0–96.5%)	222	89.6% (84.9–93.0%)	201	99.5% (97.2–99.9%)	217	93.1% (88.9–95.8%)
Homogeneous	PPA	54	81.5% (69.2–89.6%)	56	69.6% (56.7–80.1%)	49	91.8% (80.8–96.8%)	54	75.9% (63.1–85.4%)
	NPA	189	100% (98.0–100%)	187	98.9% (96.2–99.7%)	194	98.5 (95.6–99.5%)	189	99.5% (97.1–99.9%)
Speckled	PPA	45	91.1% (79.3–96.5%)	47	72.3% (58.2–83.1%)	53	83.0% (70.8–90.8%)	45	86.7% (73.8–93.7%)

Positive and negative pattern agreement on HEp-2 Elite IFA substrate, compared to predicate for individual readers (Readers 1–3), and for consensus read of 2/3 readers. *n* values depict samples positive or negative by the predicate device.									
Pattern		HEp-2 Elite IFA v Predicate ANA IFA							
		Reader 1		Reader 2		Reader 3		Consensus (≥2/3 Readers)	
	NPA	198	99.5% (97.2–99.9%)	196	98.0% (94.9–99.2%)	190	96.3% (92.6–98.2%)	198	99.5% (97.2–99.9%)

*denominator values for "all" pattern reads are greater than 243, as these represent all pattern calls, irrespective of pattern multiplicity. e.g. samples with two patterns are counted twice

** see DFS(70) pattern agreement tables below, for more detailed analysis

To specifically represent the DFS70 pattern agreement data in more detail, the data in the Method Comparison table above are also depicted in the following 2×2 contingency tables, with PPA/NPA calculations, including 95% CI:

Reader 1 DFS(70) pattern agreement on HEp-2 Elite IFA substrate, compared to predicate for individual readers					
		Predicate			
		DFS(+)	DFS(–)		
HEp-2 Elite	DFS70(+)	26	13	39	PPA: 100% (87.7–100%) NPA: 94.0% (90.0–96.5%)
	DFS70(–)	0	204	204	
		26	217	243	
Reader 2 DFS(70) Pattern Agreement					
		Predicate			
		DFS(+)	DFS(–)		
HEp-2 Elite	DFS70(+)	20	23	43	PPA: 95.2% (77.3–99.2%) NPA: 89.6% (84.9–93.0%)
	DFS70(–)	1	199	200	
		21	222	243	
Reader 3 DFS(70) Pattern Agreement					
		Predicate			
		DFS(+)	DFS(–)		
HEp-2 Elite	DFS70(+)	42	1	43	PPA: 100% (91.6–100%) NPA: 99.5% (97.2–99.9%)
	DFS70(–)	0	200	200	
		42	201	243	

From these calculations, several samples were called DFS(–) on the predicate, but DFS70(+) on the HEp-2 Elite IFA substrate by Readers 1 and 2. Among these samples that were called DFS(–) on the predicate, but DFS70(+) on the HEp-2 Elite IFA substrate, approximately similar numbers were called as Homogeneous or Speckled on the predicate and negative for Homogeneous or Speckled on the Hep-2

Elite IFA.: Reader 1 Homogeneous Predicate(+)Elite(–) = 10; Reader 2 Homogeneous Predicate(+)Elite(–) = 17; Reader 2 Speckled Predicate(+)Elite(–) = 13.

In addition, pattern identity calls were compared between individual readers on the HEp-2 Elite IFA device and presented in the table below:

Positive and negative pattern agreement on HEp-2 Elite IFA substrate, in pairwise comparisons of three readers. *n* values depict samples positive or negative by both readers.

Pattern		Reader 1 v Reader 2		Reader 1 v Reader 3		Reader 2 v Reader 3	
		n	% (95% CI)	*n*	% (95% CI)	*n*	% (95% CI)
All*	PPA	181	92.1% (87.2–95.2%)	179	73.8% (67.6–79.1%)	173	72.4% (66.2–77.9%)
	NPA	81	78.8% (69.0–86.2%)	103	73.8% (61.6–83.2%)	106	77.6% (65.3–86.4%)
DFS70	PPA	43	88.4% (75.5–94.9%)	43	86.1% (72.7–94.5%)	43	88.4% (75.5–94.9%)
	NPA	200	99.5% (97.2–99.9%)	200	99.0% (96.4–99.7%)	200	97.5% (94.3–98.9%)
Homogeneous	PPA	41	97.6% (87.4–99.6%)	48	83.3% (70.4–91.3%)	48	83.3% (70.4–91.3%)
	NPA	202	98.0% (95.0–99.2%)	195	97.9% (94.8–99.2%)	195	99.5% (97.2–99.9%)
Speckled	PPA	38	100% (90.8–100%)	51	70.6% (57.0–81.3%)	51	66.7% (53.0–78.0%)
	NPA	205	98.0% (95.1–99.2%)	192	96.9% (93.4–98.6%)	192	97.9% (94.8–99.2%)

*denominator values for "all" pattern reads are greater than 243, as these represent all pattern calls, irrespective of pattern multiplicity. e.g. samples with two patterns are counted twice

b. Matrix comparison:

Not applicable; serum is the only sample matrix.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

Tests for anti-nuclear antibodies aid in the diagnosis of SLE among other systemic autoimmune rheumatic diseases (SARD). A total of 256 diagnosed SARD samples were tested on the HEp-2 ELITE IFA. Non-ANA-associated disease conditions, including non-SARD autoimmune diseases, inflammatory diseases, cancer, and infectious diseases may be included in the intended use differential diagnosis for ANA. For comparison to the SARD sample cohort above, 254 samples of non-SARD autoimmune disease controls, 13 inflammatory disease controls, 57 cancer, and 173 samples from infectious disease cases were studied on the HEp-2 ELITE IFA.

Sensitivity and specificity were calculated from these 753 total samples as below:

SARD diseases and controls tested by the HEp-2 Elite				
		SARD		
		(+)	**(–)**	
HEp-2 Elite	**ANA(+)**	200	124	324
	ANA(–)	56	373	429
		256	497	**753**

Sensitivity (95% CI): 78.1% (72.7 – 82.8%)
Specificity (95% CI):75.1% (71.1 – 78.7%)

Performance of the HEp-2 Elite IFA for individual diseases are given for individual diagnostic categories below:

Reported ANA-positive *n*, and percent positive on HEp-2 Elite IFA with disease samples			
Disease/Indication	**Total**	**Positive**	
	n	***n***	**%**
ANA-associated SARD samples	**256**	**200**	**78.1**
Autoimmune hepatitis	13	6	46.2%
Antiphospholipid syndrome	15	7	46.7%
Drug-induced lupus	12	11	91.7%
Mixed connective tissue disease	10	7	70%
Myositis	36	16	44.4%
Sjögren's syndrome*	65	62	95.3%
Systemic lupus erythematosus*	78	74	94.9%
Systemic sclerosis*	27	17	63.0%
Autoimmune disease controls	**254**	**90**	**35.4%**
ANCA-associated vasculitis	20	1	5%
Celiac disease	10	1	10%
Crohn's disease	25	6	24%
Autoimmune thyroiditis	20	1	5%
Pernicious anemia	20	2	10%
Primary biliary cholangitis	25	17	68%
Psoriasis	20	2	10%
Raynaud's syndrome, primary	4	2	50%
Rheumatoid arthritis*	87	52	59.8%
Ulcerative colitis	23	6	26.1%
Inflammatory disease controls	**13**	**2**	**15.4%**
Atopic dermatitis	13	2	15.4%
Cancer	**57**	**3**	**5.3%**
Breast cancer	8	1	12.5%
Colorectal cancer	10	1	10%
Ovarian cancer	10	0	0%
Pancreatic cancer	9	0	0%
Prostate cancer	20	1	5%
Infectious disease controls	**173**	**29**	**16.8%**

Reported ANA-positive *n*, and percent positive on HEp-2 Elite IFA with disease samples			
Disease/Indication	**Total**	**Positive**	
	n	***n***	**%**
Cytomegalovirus	20	1	5%
Hepatitis B virus	20	1	5%
Hepatitis C virus*	11	4	36.4%
HIV	20	2	10%
Herpes simplex virus	20	2	10%
Lyme disease	12	6	50%
Mononucleosis	10	2	20%
Rubella	20	4	20%
Syphillis	20	2	10%
Toxoplasmosis	20	5	25%
Total:	**753**	**324**	**43.0%**

*cohort includes secondary Raynaud's syndrome sample(s), subordinate to primary diagnosis

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

Not applicable

4. Clinical cut-off

See Assay cut-off, above

5. Expected values/Reference range:

A cohort of 128 normal human serum samples was tested on the HEp-2 Elite IFA at the 1:40 initial dilution. Qualitative results demonstrating ANA prevalence in reference populations for this study are below:

Reported ANA-positive *n*, and percent positive on HEp-2 Elite IFA on normal human samples			
Patient Status	**Total**	**Positive**	
	n	***n***	**%**
normal human sera	128	7	5.5%

Of the seven ANA-positive samples, three were DFS(+), two were AMA(+), and two were centromere(+).

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable.

O. Conclusion:

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