510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION MEMORANDUM

A. 510(k) Number:

K180202

B. Purpose for Submission:

New Assay

C. Measurand:

Human serum IgG autoantibodies to anti-mitochondrial (AMA), anti-parietal cell (APCA), and anti-smooth muscle (ASMA)

D. Type of Test:

Qualitative and/or Semi-quantitative indirect immunofluorescence; manual or semi-automated

E. Applicant:

Immuno Concepts, N.A., Ltd.

F. Proprietary and Established Names:

Histofluor Rodent LKS Fluorescent Antibody Test System

Image Navigator by Immuno Concepts

G. Regulatory Information:

1. Regulation section:

   21 CFR §866.5660 – Multiple autoantibodies immunological test system (assay)
   21 CFR §866.4750 – Automated indirect immunofluorescence microscope and software-assisted system (instrument)

2. Classification:

   Class II (assay and instrument)

3. Product code:

   DBL, Multiple autoantibodies immunological test system
   PIV, Automated indirect immunofluorescent microscope and software-assisted system for clinical use

4. Panel:

   Immunology (82)

H. Intended Use:

1. Intended use(s):

   This is an indirect fluorescent antibody test for the qualitative or semi-quantitative detection of IgG autoantibodies in human serum by manual fluorescent microscopy or with the Image Navigator Fluorescence Semi-Automated Microscope. This test system is to be used as an aid in the detection of anti-mitochondrial (AMA), anti-parietal cell (APCA), and anti-smooth muscle (ASMA) antibodies associated with Type 1 Autoimmune Hepatitis, Primary Biliary Cholangitis, and Pernicious Anemia/Autoimmune Gastritis in conjunction with other laboratory and clinical findings. A trained operator must confirm results generated with the Image Navigator semi-automated device and software.

2. Indication(s) for use:

   Same as Intended Use

3. Special conditions for use statement:

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

4. Special instrument requirements:

   Optional use on either the semi-automated Image Navigator fluorescence microscopy system, or manual fluorescence microscopy. Immuno Concepts Image Navigator is a semi-automated system consisting of a fluorescent microscope and the software does not make result recommendations for the LKS assay. A trained operator must interpret the results.

I. Device Description:

The indirect fluorescent antibody kit to be used for detection of multiple autoantibodies is a

device for use either in a conventional immunofluorescent assay or by analysis with the Image Navigator. The same kit, with no modifications, is used for both conventional and Image Navigator analysis.

Histofluor Rodent LKS Fluorescent Antibody Test System Device Description		
Kit Component	Part Number	Description
Substrate Slides	12004-02: 4-well mouse tissues	Multi-well 4-5 μm fixed sections of composite rodent liver, kidney, and stomach tissues on glass slides
	12008-02: 8-well mouse tissues	
	12004-03: 4-well rat tissues	
	12008-03: 8-well rat tissues	
AMA positive control	12021-02	1.0 mL positive control human serum with IgG antibodies specific to mitochondrial antigens
Negative control	12031	1.0 mL human control serum
Fluorescent antibody reagent (conjugate)	9.0 mL: 12009-02 23 mL: 12075-02	goat-anti-human IgG conjugated to FITC
Optional control reagents		
APCA positive control	12022-02	0.5 mL positive control human serum with IgG antibodies specific to gastric parietal cell antigens
ASMA positive control	12023-02	0.5 mL positive control human serum with IgG antibodies specific to smooth muscle antigens
Optional titratable control reagents		
AMA titratable control	12261-02	0.25 mL undiluted positive control human serum with IgG antibodies specific to mitochondrial antigens
APCA titratable control	12262-02	0.25 mL undiluted positive control human serum with IgG antibodies specific to gastric parietal cell antigens
ASMA titratable control	12263-02	0.25 mL undiluted positive control human serum with IgG antibodies specific to smooth muscle antigens
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	10 24×64 mm No. 1 glass coverslips

Image Navigator is an automated scanning microscope and image presentation system. The

system is comprised of a computer, monitor, keyboard, mouse, barcode readers, installed software, microscope, and digital camera. The automated microscope includes a motorized stage, autofocus drive, brightfield lamp, LED fluorescence lamp, eyepieces, condenser, and camera adapter. Images captured by the digital camera are transferred to the computer for entry into a file appropriate to the patient identification, and subsequent processing by the software. For this assay, Immuno Concepts Image Navigator does not make result recommendations. A trained operator must interpret the results.

J. Substantial Equivalence Information:

1. Predicate device names:
   MeDiCa IIF Multiple Antibody Test Kit (assay)
   Image Navigator by Immuno Concepts (instrument)

2. Predicate 510(k) numbers:
   K831100 (assay)
   K160265 (instrument)

3. Comparison with predicate:

Similarities		
Item	Device (K180202)	Assay Predicate (K831100)
Methodology	Indirect immunofluorescence (IFA)	Same
Substrate	Rodent (rat, mouse) liver + kidney + stomach	Same
Starting Dilution	1:20	Same
Assay Cutoff	1:20	Same
Sample Matrix	Serum	Same
Conjugate	Goat anti-human IgG FITC	Same
Results	qualitative, pattern, semi-quantitative, titer	Same
Item	Device (K180202)	Instrument Predicate (K160265)
Software	Automated image acquisition and digital display for semi-automated image interpretation	Same

Differences		
Item	Device (K180202)	Assay Predicate (K831100)
Intended Use	This is an indirect fluorescent antibody test for the semi-quantitative detection of IgG antibodies in human serum by manual fluorescent microscopy or with the Image Navigator	MeDiCa Indirect Immunofluorescence (IIF) Antibody Test Kits are used for the qualitative and semi-quantitative detection of specific Ig classes of autoantibodies in

Differences		
Item	Device (K180202)	Assay Predicate (K831100)
	Fluorescence Semi-Automated Microscope. This test system is to be used as an aid in the detection of antibodies associated with autoimmune diseases in conjunction with other laboratory and clinical findings. A trained operator must confirm results generated with the Image Navigator semi-automated device and software.	human serum as an aid in the diagnosis of certain pathologies.
Measurands	Human serum IgG autoantibodies: anti-mitochondrial (AMA) anti-parietal cell (APCA) anti-smooth muscle (ASMA)	AMA, APCA, ASMA, and anti-nuclear antibodies (ANA)
Instrument	The Image Navigator is an optional automated microscope designed to acquire and display images for the user	Conventional fluorescent microscopy
Item	Device (K180202)	Instrument Predicate (K160265)
Software	Image Navigator does not provide recommended qualitative outcome (i.e. positive or negative) to the user following image acquisition	Image Navigator makes recommendations to the user for qualitative outcomes (i.e. positive or negative) following image acquisition
Image Acquistion & Processing	Capture of 16 images of 100× total magnification, stitched into single image	Capture of 4 images at 200× total magnification, stitched into single image
Image Evaluation	Operator-dependent	Software recommendation of (+) or (–) value to operator

K. Standard/Guidance Document Referenced:

Org	Standard ID	Version	Date	Title
CLSI	EP07	A2	Dec 2002	Interference Testing in Clinical Chemistry

L. Test Principle:

The Immuno Concepts Histofluor Rodent LKS Test System uses the indirect fluorescent antibody technique. Patient samples are incubated on substrate slides with composite liver/kidney/stomach (LKS) tissue sections from mouse or rat to allow specific binding of autoantibodies to cell components. If autoantibodies are present, 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 antigen distribution within cells or tissues. If the sample is negative for autoantibodies, the substrate will not show a clearly discernible pattern of fluorescence.

M. Performance Characteristics:

1. Analytical performance:

a. *Precision/Reproducibility:*

Multi-site reproducibility studies were performed on 13 patient serum samples representing four each of the three patterns (AMA, APCA, ASMA) and one negative sample. For each pattern, the samples were low titer, medium titer, and high titer. For both manual and semi-automated Image Navigator reading modes, for both substrate species, two users/site at three sites read each sample in triplicate over 10 runs for a total of 312 datapoints.

Based on these outcomes, agreement measures for endpoint titer and pattern were within pre-specified acceptance criteria; the lower confidence interval bounds for all pairwise comparisons on each substrate, in either reading mode were all ≥88.6%. Agreement measures for endpoint titer and pattern identification between users and between sites are presented in the tables below:

Endpoint Titer Agreement between Readers:

Reproducibilty: Mouse, Manual 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		97.8% (95.6–99.0%)	97.2% (94.9–98.6%)	90.3% (86.8–93.0%)	96.7% (94.2–98.1%)	98.3% (96.3–99.3%)
	User 2			99.4% (97.9–100%)	95.6% (92.9–97.3%)	98.1% (96.0–99.1%)	98.9% (97.1–99.7%)
Site 2	User 1				94.2% (91.2–96.2%)	97.8% (95.6–99.0%)	98.6% (96.7–99.5%)
	User 2					92.2% (89.0–94.6%)	91.9% (88.6–94.4%)
Site 3	User 1						98.1% (96.0–99.1%)
	User 2						

Reproducibilty: Mouse, Semi-Automated – % 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		98.6% (96.7–99.5%)	98.9% (97.1–99.7%)	95.6% (92.9–97.3%)	98.6% (96.7–99.5%)	99.4% (97.9–100%)
	User 2			98.6% (96.7–99.5%)	95.0% (92.2–96.9%)	98.6% (96.7–99.5%)	98.6% (96.7–99.5%)
Site 2	User 1				96.7% (94.2–98.1%)	98.6% (96.7–99.5%)	99.4% (97.9–100%)
	User 2					93.6% (90.6–95.8%)	95.6% (92.9–97.3%)
Site 3	User 1						99.4% (97.9–100%)
	User 2						

Reproducibilty: Rat, Manual 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		96.1% (93.5–97.7%)	97.2% (94.9–98.6%)	96.1% (93.5–97.7%)	96.7% (94.2–98.1%)	95.8% (93.2–97.5%)
	User 2			97.2% (94.9–98.6%)	95.6% (92.9–97.3%)	96.7% (94.2–98.1%)	95.0% (92.2–96.9%)
Site 2	User 1				97.2% (94.9–98.6%)	98.3% (96.3–99.3%)	98.3% (96.3–99.3%)
	User 2					97.8% (95.6–99.0%)	94.7% (91.9–96.6%)
Site 3	User 1						95.8% (93.2–97.5%)
	User 2						

Reproducibilty: Rat, Semi-Automated – % 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		95.8% (93.2–97.5%)	95.8% (93.2–97.5%)	95.6% (92.9–97.3%)	98.1% (96.0–99.1%)	95.0% (92.2–96.9%)
	User 2			96.4% (93.9–97.9%)	94.7% (91.9–96.6%)	96.7% (94.2–98.1%)	95.6% (92.9–97.3%)
Site 2	User 1				96.4% (93.9–97.9%)	98.3% (96.3–99.3%)	98.1% (96.0–99.1%)
	User 2					96.9% (94.6–98.4%)	95.3% (92.5–97.1%)
Site 3	User 1						97.2% (94.9–98.6%)
	User 2						

Pattern Agreement between Readers:

Reproducibilty: Mouse, Manual Mode – % Pattern Agreement, 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		100% (98.7–100%)	100% (98.7–100%)	99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
	User 2			100% (98.7–100%)	99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
Site 2	User 1				99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
	User 2					99.7% (98.3–99.9%)	99.7% (98.3–99.9%)
Site 3	User 1						100% (98.7–100%)
	User 2						

Reproducibilty: Mouse, Semi-Automated – % Pattern Agreement, 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		100% (98.7–100%)	100% (98.7–100%)	99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
	User 2			100% (98.7–100%)	99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
Site 2	User 1				99.7% (98.3–99.9%)	100% (98.7–100%)	100% (98.7–100%)
	User 2					99.7% (98.3–99.9%)	99.7% (98.3–99.9%)
Site 3	User 1						100% (98.7–100%)
	User 2						

Reproducibilty: Rat, Manual Mode – % Pattern Agreement, 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		100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
	User 2			100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
Site 2	User 1				100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
	User 2					100% (98.7–100%)	100% (98.7–100%)
Site 3	User 1						100% (98.7–100%)
	User 2						

Reproducibilty: Rat, Semi-Automated – % Pattern Agreement, 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		100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
	User 2			100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
Site 2	User 1				100% (98.7–100%)	100% (98.7–100%)	100% (98.7–100%)
	User 2					100% (98.7–100%)	100% (98.7–100%)
Site 3	User 1						100% (98.7–100%)
	User 2						

Lot-to-lot precision was evaluated using both manual and semi-automated reading modes using three lots of each substrate (mouse or rat) × 32 samples × three readers. Samples were selected to represent all three patterns (i.e. AMA, APCA, ASMA), across the range of titers.

Lot-to-lot precision (% with 95% CI)								
Substrate	Measure	*n*	Manual			Semi-automated		
			Lot 1 v 2	Lot 1 v 3	Lot 2 v 3	Lot 1 v 2	Lot 1 v 3	Lot 2 v 3
Mouse	PPA	30	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)
	NPA	2	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)
	Pattern	30	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)	100% (86.5–100%)
	±1 Titer	30	96.7% (81.9–100%)	96.7% (81.9–100%)	96.7% (81.9–100%)	90.0% (73.6–97.3%)	96.7% (81.9–100%)	100% (86.5–100%)
Rat	PPA	30	100% (86.5–100%)	96.7% (81.9–100%)	96.7% (81.9–100%)	100% (86.5–100%)	96.7% (81.9–100%)	96.7% (81.9–100%)
	NPA	2	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)	100% (29.0–100%)
	Pattern	30	100% (86.5–100%)	96.7% (81.9–100%)	96.7% (81.9–100%)	100% (86.5–100%)	96.7% (81.9–100%)	96.7% (81.9–100%)
	±1 Titer	30	93.3% (77.6–99.2%)	96.7% (81.9–100%)	93.3% (77.6–99.2%)	100% (86.5–100%)	93.3% (77.6–99.2%)	90.0% (73.6–97.3%)

b. *Linearity/assay reportable range:*

To assess endpoint titration, one sample from each of the target patterns (AMA, APCA, and ASMA) was evaluated on Histofluor Mouse and Rat Tissue slides. The sample was assayed in duplicate and analyzed by two readers using both conventional and semi-automated reading methods. A consistent pattern was found throughout the

titer to the endpoint and a consistent titer endpoint was found between readers and methods (within ±1 titer).

Mouse Substrate:

Expected Pattern	Replicate	Manual				Semi-Automated			
		Reader 1		Reader 2		Reader 1		Reader 2	
		Pattern	Titer	Pattern	Titer	Pattern	Titer	Pattern	Titer
ASMA	1	ASMA	1:640	ASMA	1:320	ASMA	1:320	ASMA	1:320
AMA	1	AMA	1:80	AMA	1:80	AMA	1:80	AMA	1:80
APCA	1	APCA	1:80	APCA	1:80	APCA	1:80	APCA	1:80
ASMA	2	ASMA	1:640	ASMA	1:320	ASMA	1:320	ASMA	1:320
AMA	2	AMA	1:160	AMA	1:80	AMA	1:80	AMA	1:80
APCA	2	APCA	1:80	APCA	1:80	APCA	1:80	APCA	1:80

Rat Substrate:

Expected Pattern	Replicate	Manual				Semi-Automated			
		Reader 1		Reader 2		Reader 1		Reader 2	
		Pattern	Titer	Pattern	Titer	Pattern	Titer	Pattern	Titer
ASMA	1	ASMA	1:640	ASMA	1:640	ASMA	1:320	ASMA	1:320
AMA	1	AMA	1:160	AMA	1:80	AMA	1:80	AMA	1:80
APCA	1	APCA	1:80	APCA	1:80	APCA	1:80	APCA	1:80
ASMA	2	ASMA	1:640	ASMA	1:320	ASMA	1:320	ASMA	1:320
AMA	2	AMA	1:160	AMA	1:80	AMA	1:160	AMA	1:80
APCA	2	APCA	1:80	APCA	1:80	APCA	1:80	APCA	1:80

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

Recognized consensus, traceable reference standards are not available for AMA, APCA, or ASMA autoantibodies.

Real-time stability studies demonstrated shelf-life of the Histofluor rodent LKS assays to a minimum 12 months at 2–10° C. Open-kit stability studies supported in-use stability to a minimum of 8 weeks at at 2–10° C. Stability studies evaluated comparable qualitative (positive/negative), pattern identification, and endpoint titer (±1 dilution) outcomes.

d. *Detection limit:*

For semi-quantitative IFA, detection limits are not applicable.

e. *Analytical specificity:*

<u>Cross-Reactivity</u>

Potentially cross-reactive disease sera were tested for LKS autoantibodies using the

Histofluor LKS IFA including 121 samples from other autoimmune diseases (autoimmune thyroiditis, celiac disease, Crohn's and IBS, type 1 diabetes, systemic sclerosis, Sjögren's syndrome, systemic lupus erythematosus, rheumatoid arthritis, and autoimmune vasculitis); lymphoma; cirrhosis from causes other than AIH or PBC; *Helicobacter pylori* infection; HBV and HCV. 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 claimed conformance to CLSI EP07-A2. Three specimens were tested, one negative, one medium-titer positive (1:320), and one high-titer positive (1:1280) in triplicate for three concentrations of each of five interferents. Interferents were spiked by predilution and addition of 10% (v/v) of final total sample volume. Controls were diluted with unspiked 10% (v/v) diluent.

All samples were tested on mouse LKS slides and evaluated by both manual and semi-automated reading modes by three operators. Fluorescence of samples containing the interfering substances were within ± one intensity grade of the control samples with both reading modes. No interference was detected with bilirubin up to 10 mg/dL, hemoglobin up to 200 mg/dL, triglycerides up to 1000 mg/dL, cholesterol up to 230 mg/dL, ampicillin (as a representative cholestatic) up to 152 μM, omeprazole (as a representative proton pump inhibitor) up to 17.4 μM, and rheumatoid factor IgM up to 60 units.

f. *Assay cut-off:*

Clinical performance studies evaluated a 1:20 cutoff.

2. Comparison studies:

a. *Method comparison:*

One-hundred seventy clinical patient samples (se Section M.3 below) were tested in parallel on the Histofluor LKS substrates and the predicate assay at three sites for consensus adjudication. Positive percent agreement (PPA) and negative percent agreement (NPA) values were calculated, with 95% confidence intervals. Agreement of pattern identity calls was also evaluated for positive samples, as was agreement of endpoint titer, within agreement defined as within ±1 dilution.

Comparison results are presented in the following table, comparing the Histofluor LKS to the predicate using manual, conventional fluoromicroscopy methods; and the manual, conventional fluoromicroscopy to the software-assisted semi-automated reading mode for the Histofluor LKS substrates:

		Method Comparisons				
		n	Histofluor LKS v Predicate Manual		Histofluor LKS Manual v Semi-Automated	
			% Agreement	(95% CI)	% Agreement	(95% CI)
Mouse	PPA	36	94.4%	(80.9–99.4%)	100%	(88.5–100%)
	NPA	137	98.5%	(94.5–99.9%)	100%	(96.7–100%)
	Pattern[1]	36	94.4%	(80.9–99.4%)	100%	(88.5–100%)
	±1 Titer	36	80.6%	(64.7–90.6%)	100%	(88.5–100%)
Rat	PPA	39	92.3%	(79.0–98.1%)	100%	(88.5–100%)
	NPA	134	97.8%	(93.4–99.5%)	100%	(96.6–100%)
	Pattern[1]	39	92.3%	(79.0–98.1%)	100%	(87.9–100%)
	±1 Titer	39	76.9%	(61.5–87.6%)	100%	(87.9–100%)

[1] Pattern identity calls represent agreement of qualitatively positive (+) samples

b. *Matrix comparison:*

Serum is the only claimed, validated sample matrix.

3. Clinical studies:

Forty-nine samples from Intended Use indications (i.e. type 1 autoimmune hepatitis [AIH-1], pernicious anemia/autoimmune gastritis [PA/AG], and primary biliary cholangitis [PBC]) and 121 differential diagnosis samples described in Section M.1.e were tested on the Histofluor Rodent LKS IFA. Sensitivity and specificity were calculated from these 170 total samples and summarized just below, with a more detailed analysis in Section M.3.a and b. below.

Disease and control samples tested by the Histofluor Mouse LKS in manual reading mode				
		AIH, PA/AG, PBC		
		(+)	**(–)**	
Histofluor Mouse LKS	**LKS(+)**	29	7	36
	LKS(–)	20	114	134
		49	121	**170**

Sensitivity (95% CI): 59.2% (45.2 – 71.8%)
Specificity (95% CI): 94.2% (88.3 – 97.4%)

Disease and control samples tested by the Histofluor Rat LKS in manual mode				
		AIH, PA/AG, PBC		
		(+)	**(–)**	
Histofluor Rat LKS	**LKS(+)**	30	7	37
	LKS(–)	19	114	133
		49	121	**170**

Sensitivity (95% CI): 61.2% (47.2 – 73.6%)
Specificity (95% CI): 94.2% (88.5 – 97.2%)

Values were comparable to results obtained using the predicate device.

a. *Clinical Sensitivity:*

A total of 49 samples from the intended use indications were tested at three sites and read by three operators at each site.

	Clinical Sensitivity (% Se)					
	Disease	*n*	Manual		Semi-automated	
			% Se	(95% CI)	% Se	(95% CI)
Mouse	All	49	59.2%	(45.3–71.8%)	59.2%	(45.2–71.8%)
	Autoimmune Hepatitis[1]	19	47.4%	(27.3–68.3%)	47.4%	(27.3–68.3%)
	Pernicious Anemia/ Autoimmune Gastritis[2]	12	41.7%	(19.3–68.1%)	41.7%	(19.3–68.1%)
	Primary Biliary Cholangitis	18	83.3%	(60.0–95.0%)	83.3%	(60.0–95.0%)
Rat	All	49	61.2%	(47.2–73.6%)	61.2%	(47.2–73.6%)
	Autoimmune Hepatitis[1]	19	47.4%	(27.3–68.3%)	47.4%	(27.3–68.3%)
	Pernicious Anemia/ Autoimmune Gastritis[2]	12	50.0%	(25.4–74.6%)	50.0%	(25.4–74.6%)
	Primary Biliary Cholangitis	18	83.3%	(60.0–95.0%)	83.3%	(60.0–95.0%)
	[1] AIH positives represent both ASMA and AMA patterns [2] cohort combines patients with either pernicious anemia or autoimmune gastritis diagnoses; represent both APCA and ASMA patterns					

b. *Clinical specificity:*

One-hundred twenty one clinically characterized samples were tested at three sites and read by three operators at each. Values represent consensus adjudication (>2/3) reads.

	Clinical Specificity (% Sp)					
	Disease	*n*	Manual		Semi-automated	
			% Sp	(95% CI)	% Sp	(95% CI)
Mouse	Autoimmune thyroid disease[3]	10	94.2%	(88.3–97.4%)	94.2%	(88.3–97.4%)
	Lymphoma	9				
	Celiac disease	10				
	Cirrhosis[4]	2				
	Helicobacter pylori	10				
	IBS, Crohn's disease	10				
	Type 1 diabetes	10				
	HBV, HCV	10				
	Systemic sclerosis	10				
	Sjögren's syndrome	10				

	Clinical Specificity (% Sp)					
	Disease	*n*	Manual		Semi-automated	
			% Sp	(95% CI)	% Sp	(95% CI)
	Systemic lupus erythematosus	10				
	Rheumatoid arthritis	10				
	Autoimmune vasculitis[5]	10				
Rat	Autoimmune thyroid disease[3]	10	93.4%	(87.3–96.8%)	93.4%	(87.3–96.8%)
	Lymphoma	9				
	Celiac disease	10				
	Cirrhosis[4]	2				
	Helicobacter pylori	10				
	IBS, Crohn's disease	10				
	Type 1 diabetes	10				
	HBV, HCV	10				
	Systemic sclerosis	10				
	Sjögren's syndrome	10				
	Systemic lupus erythematosus	10				
	Rheumatoid arthritis	10				
	Autoimmune vasculitis[5]	10				
	[3] diagnostic category includes Hashimoto's/lymphocytic and Graves' thyroiditis [4] cohort excludes AIH, PBC patients [5] diagnostic category includes biopsy-verified samples					

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

Not applicable

4. Clinical cut-off:

Same as assay cutoff (M.1.f)

5. Expected values/Reference range:

Samples from a cohort of from 120 apparently healthy subjects (84 females and 36 males) were tested at 3 sites by two readers:

	Reference Range					
	Dx	*n*	Manual		Semi-automated	
			% (+)	(95% CI)	% (+)	(95% CI)
Mouse	"apparently healthy subjects"	120	1.7%	(0.1–6.2%)	1.7%	(0.1–6.2%)
Rat			1.7%	(0.1–6.2%)	1.7%	(0.1–6.2%)

The 2/120 positive samples in this study produced an APCA pattern at > 1:160 titer.

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Parts 801 and 809, as applicable, and the special controls for this device type under 21 CFR 866.5180.

O. Conclusion:

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