510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:
k060157

B. Purpose for Submission:
New Devices

C. Measurand:
Anti-Endomysial Antibody

D. Type of Test:
Qualitative and semi-quantitative, indirect immunofluorescence (IFA)

E. Applicant:
IMMCO Diagnostics, Inc.

F. Proprietary and Established Names:
ImmuGlo™ Anti-Endomysial IFA

G. Regulatory Information:

1. Regulation section:
   21 CFR 866.5660, Multiple Autoantibodies Immunological test system
2. Classification:
   II
3. Product code:
   MVM, Autoantibodies, Endomysial (Tissue Transglutaminase)
4. Panel:
   Immunology 82

H. Intended Use:

1. Intended use(s):
   An indirect immunofluorescence antibody test for the qualitative and semi-quantitative detection of endomysial antibodies (EMA of IgA or IgG) in human serum as an aid in the diagnosis of celiac disease and *dermatitis herpetiformis* in combination with other clinical and other laboratory findings.
2. Indication(s) for use:
   Same as intended use.
3. Special conditions for use statement(s):
   For prescription use only.
4. Special instrument requirements:
   Fluorescence microscope

I. Device Description:
Each device contains the following: 8x6-well-Primate Distal Esophagus Substrate slides, EMA positive and negative controls, goat anti-human FITC IgA and IgG conjugates with Evan's Blue Counter stain, buffered diluent, phosphate buffered saline, mounting medium, cover slips.

J. Substantial Equivalence Information:

1. Predicate device name(s):
   ImmuGlo™ EMA IFA System with Primate Smooth Muscle
   ImmuLisa™ tTgG IgG ELISA
2. Predicate 510(k) number:

k912551 ImmuGlo™ EMA IFA
k040095 ImmuLisa™ tTG IgG ELISA

3. Comparison with predicate:

Similarities		
Item	New Device	Predicate Device
Intended use	To aid in the diagnosis of Celiac Disease (CD) and dermatitis herpetiformis	Same
Anti-EMA IgA and IgG assay format	Qualitative and semi-quantitative	Same
Anti-EMA IgA and IgG Controls	Positive and Negative Controls	Same
Anti- EMA IgA technology	IFA	Same
Anti-EMA IgA (IFA) platform	8x6 well slides	Same
Anti-EMA IgA (IFA) sample dilution	1:2.5	Same
Anti-EMA IgA (IFA): Incubation time	30-30 minute	Same
Anti-EMA IgA (IFA): Wash Step	Two 10 minute wash	Same
Anti-EMA IgA (IFA) Cut-off	2.5 Titer	Same
Anti-EMA IgA semi-quantitative result	End point Titer	Same

Differences		
Item	New Device	Predicate
Anti-EMA antigen	IgA and IgG (IFA) : Primate smooth muscle from distal esophagus	IgA (IFA): Primate smooth muscle from the bladder IgG (ELISA): Recombinant human tTG
Anti-EMA Conjugate	FITC IgA and FITC IgG	IgA (IFA): FITC polyvalent IgG (ELISA): Alkaline phosphatase
Anti-EMA IgG technology	IFA	ELISA
Anti-EMA IgG platform	8x6 well slides	ELISA: 12x8 Microwells
Anti-EMA IgG sample dilution	1:2.5	ELISA: 1:51

Differences		
Item	New Device	Predicate
Anti-EMA IgG: calibrators	Not Applicable	ELISA: 4 level calibrators
Anti-EMA IgG: substrate	Not Applicable	ELISA: pNPP
Anti-EMA IgG incubation times (min)	30-30	30-30-30
Anti-EMA IgG: Wash step	Two 10 minute wash	Two 4X wash
Anti-EMA IgG: reading	IFA: Fluorescence microscope at 200X or greater magnification	ELISA: Spectrophotometer: OD at 405/620 nm
Anti-EMA IgG cut-off	2.5 Titer	ELISA: 20 EU/mL
Anti-EMA IgG semi-quantitative result	Endpoint Titer	ELISA: Negative: <20 EU/mL Indeterminate (Borderline): 20-25 EU/mL Positive: >25 EU/mL

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

None referenced.

L. Test Principle:

Using this indirect immunofluorescence method, patient serum is incubated on tissue sections to allow binding of antibodies to the substrate. Any antibodies not bound are removed by rinsing. Bound antibodies of the IgA or IgG class are detected by incubation of the substrate with fluorescein-labeled, anti-human immunoglobulin conjugates IgA or IgG respectively. Reactions are observed under a fluorescence microscope equipped with appropriate filters. The presence of EMA is demonstrated by an apple green fluorescence of the endomysium of smooth muscle bundles especially the inner layer. When positive, the titer (the reciprocal of the highest dilution giving a positive reaction) of the antibody is then determined by testing serial dilutions.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:
   a. *Precision/Reproducibility*:

      The Anti-EMA IgA IFA intra-assay reproducibility was determined by testing three samples, i.e. low (2.5 titer), medium (20 titer), and high (1280 titer) ten times. All samples were within one titer difference. The IFA inter-assay reproducibility was determined by testing the same three samples in singlicate ten times in ten runs. All samples were within one titer difference.

      The Anti-EMA IgG IFA intra-assay reproducibility was determined by testing three samples with high titer* (1280 – 20480) ten times. All samples were within one titer difference. The IFA inter-assay reproducibility was

determined by testing the same three samples in singlicate ten times in ten runs. All samples were within one titer difference.
*Since anti-EMA IgG antibodies, when present, are generally in high titer, no patient samples close to the cut-off (usually difficult to obtain) were available for reproducibility studies.

b. *Linearity/assay reportable range:*
Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
Not applicable.

d. *Detection limit:*
Not applicable.

e. *Analytical specificity:*
Interference by endogenous substances: The package insert states that grossly hemolyzed, lipemic, or microbially contaminated samples should not be used in these assays.
Cross-reactivity: Ten rheumatoid arthritis and ten systemic lupus erythematosus specimens were tested by both Anti-EMA IgA and IgG assays. All samples were negative.

f. *Assay cut-off:*
The cut-off value of 2.5 for EMA IgA was established using a population of 43 CD patients and 294 controls. Titers of CD patients ranged from 2.5 to >80. Titers of controls were all less than 2.5.

The cut-off value of 2.5 for EMA IgG was established using a population of 78 CD patients and 73 controls. Titers of CD patients ranged from 2.5 to 10240. Titers of controls were all less than 2.5.

2. Comparison studies:

a. *Method comparison with predicate device:*
Anti- EMA IgA - testing was performed on 215 samples which included 176 CD samples. The positive percent agreement was 98.9% (174/176); the negative percent agreement was 100% (39/39); and the overall agreement was 99.1% (213/215). Results are summarized below.

		Anti-EMA IgA IFA (Primate Smooth Muscle)		
		Positive	Negative	Total
Anti-EMA-IgA IFA (Primate Distal Esophagus)	Positive	174	0	174
	Negative	2	39	41
	Total	176	39	215

Positive percent Agreement: 98.9% (174/176)
Negative percent agreement: 100% (39/39)
Overall percent agreement: 99.1% (213/215)

The Anti- EMA IgG - testing was performed on 50 samples which included

18 CD samples. The positive percent agreement was 83.3% (15/18); the negative percent agreement was 96.9% (31/32); and the overall agreement was 92% (46/50). Results are summarized below.

		ImmuLisa Anti-tTG IgG (ELISA)		
		Positive	Negative	Total
Anti-EMA IgG IFA (Primate Distal Esophagus)	Positive	15	1	16
	Negative	3	31	34
	Total	18	32	50

Positive percent Agreement: 83.3% (15/18)
Negative percent agreement: 96.9% (31/32)
Overall percent agreement: 92 % (46/50)

b. Matrix comparison:
Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and specificity:
The Anti-EMA IgA IFA clinical sensitivity and specificity study were evaluated on 60 clinically defined samples from patients with the following diagnosis: 30 Celiac Disease and 30 normal subjects. The Anti-EMA IgA IFA sensitivity was 100% (30/30) and the specificity 100% (30/30). Study results are summarized in the table below.

		Diagnosis		
		Positive	Negative	Total
Anti-EMA IgA IFA (Primate Distal Esophagus)	Positive	30	0	30
	Negative	0	30	30
	Total	30	30	60

Sensitivity: 100% (30/30)
Specificity: 100% (30/30)

The Anti-EMA IgG IFA clinical sensitivity and specificity study were evaluated on 24 clinically defined samples from patients with the following diagnosis: 14 Celiac Disease and 10 normal subjects. The Anti-EMA IgG IFA sensitivity was 100% (14/14) and the specificity 100% (10/10). Study results are summarized in the table below.

		Diagnosis		
		Positive	Negative	Total
Anti-EMA IgG IFA (Primate Distal Esophagus)	Positive	14	0	14
	Negative	0	10	10
	Total	14	10	24

Sensitivity: 100 % (14/14)
Specificity: 100 % (10/10)

b. Other clinical supportive data (when a is not applicable):
Not applicable.

4. Clinical cut-off**:**
Same as assay cut-off.
5. Expected values/Reference range:
Expected values in the normal population should be negative.

N. Proposed Labeling:

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

O. Conclusion:

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