

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY**

A. 510(k) Number:

k061408

B. Purpose for Submission:

New Device

C. Measurand:

Human Anti-Endomysial antibody (IgA)

D. Type of Test:

Qualitative and Semi-quantitative, Immunofluorescence Assay (IFA).

E. Applicant:

EUROIMMUN US LLC

F. Proprietary and Established Names:

Endomysium IFA: Esophagus (Monkey) Kit

G. Regulatory Information:

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

H. Intended Use:

1. Intended use(s):
The EUROIMMUN Endomysium IFA kit is designed for the semi-quantitative and qualitative determination of antibodies against Endomysium (EMA) in human serum. This test is used as an aid in the diagnosis of gluten-sensitive enteropathies associated with celiac disease in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
Same as Intended Use.
3. Special conditions for use statement(s):
The device is for prescription use only.
4. Special instrument requirements:
Fluorescent microscope with 100 W mercury vapor lamp or LED bluelight light source, 488 nm excitation filter, 510 nm color separator, 520 nm blocking filter, 20X objective for organ sections, and 40X objective for cell substrates.

I. Device Description:

The test system consists of BIOCHIPS with (10 or 20) frozen sections of primate (monkey) esophagus. It includes a fluorescein-labeled goat anti-human IgA, a positive and negative control, salt for PBS, Tween 20, embedding medium, cover glasses, and instruction booklet. Reagent trays for the TITERPLANE technique are required but ordered separately.

J. Substantial Equivalence Information:

1. Predicate device name(s):
The Binding Site Monkey Oesophagus IFA kit
2. Predicate 510(k) number(s):
k981209
3. Comparison with predicate:

Similarities		
Item	New Device	Predicate
Reagents	Tissue sections of primate esophagus	Same
Technology	Immunofluorescence	Same
Sample type and dilution	Serum, 1:10	Same
Assay format	Qualitative or semi-quantitative	Same
Procedure	Standard IFA technique: serum incubation with tissue sections, followed by a wash step, incubation with fluorescein-labeled anti-human globulin, wash step, embedding and reading fluorescence with a fluorescence microscope	Same

Differences		
Item	Device	Predicate
Intended Use	The EUROIMMUN Endomysium IFA kit is designed for the semi-quantitative and qualitative determination of antibodies against Endomysium (EMA) in human serum. This test is used as an aid in the diagnosis of gluten-sensitive enteropathies associated with celiac disease in conjunction with other laboratory and clinical findings.	Monkey oesophagus sections are intended for use as a substrate for the screening of antibodies to intra-epidermal antigens or basement membrane zones in human serum, as an aid in the diagnosis of pemphigus and bullous pemphigoid respectively. The sections can also be used for the detection of Endomysial antibodies as an aid in the diagnosis of celiac disease.
Conjugate	FITC goat anti-human IgA	FITC sheep anti-human IgG (heavy & light chain)
Incubation conditions	Humidity chamber not needed	Humidity chamber
Slide configuration	10 or 20 wells per slide	5 wells per slide
Sample/reagent application	Slides inverted over drops of diluted serum or reagent in the reagent tray	Diluted serum or reagent applied directly to substrate wells

Differences		
Item	Device	Predicate
Controls	1 each, positive and negative	2 positive (1 – pemphigus pattern, 1 – endomysial pattern); 1 negative
Cut-off level	1:10 dilution	Not calibrated

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

None provided.

L. Test Principle:

The Esophagus (Monkey) BIOCHIP substrates are incubated with diluted patient samples. Patient samples, controls and in separate steps conjugate and embedding medium are applied to the reaction fields of a reagent tray. The BIOCHIP Slides are then placed into the recesses of the reagent tray, where all BIOCHIPs of the slide come into contact with the fluids, and the individual reactions commence simultaneously. The fluids are confined to the recessed wells eliminating the need to use a conventional “humidity chamber.” If the reaction is positive, specific antibodies attach to the antigens on the specific substrate slide sections. In a second step, fluorescein-labeled anti-human antibodies reagent is added and if the specific antibodies are present, they are stained with the fluorescein reagent. The specific reaction patterns are then observed by fluorescence microscopy and the intensity of the specific fluorescence, as a numeric value, is called fluorescence intensity level. These values range from “0” (no specific fluorescence) to “5” (extremely strong specific fluorescence). For qualitative evaluation, a sample is considered to be positive if a titer of $\geq 1:10$ results in a positive reaction and in the semi-quantitative interpretation the endpoint titer is defined as the highest sample dilution factor for which specific fluorescence is identifiable.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Intra-assay reproducibility:

Four characterized sera samples were incubated on fixed, frozen slides in duplicate, triplicate, or quadruplicate. In semi-quantitative evaluation of staining intensity, no more than ± 1 difference in fluorescence intensity from the target values for 2/3 (66.7%) and 4/4 (100%) replicate slides for two separate samples were observed.

Inter-assay reproducibility:

Three characterized sera samples were incubated on substrate slides in duplicate, triplicate, or quadruplicate. Results of the semi-quantitative evaluation of staining intensity showed a deviation of no more than ± 1 from the target value for two samples.

Inter-lot reproducibility: Thirteen characterized sera were tested with different slide lots within a specific time period to estimate any deviations between individual lots. Semi-quantitative evaluation of staining intensity demonstrated a deviation in fluorescence intensity, from the target value, of no more than ± 1 for four samples.

- b. *Linearity/assay reportable range:*
Linearity not assessed. The dilution starting point for the measurement system is 1:10. Samples can be further diluted by a factor of 10. There is no upper limit to the measurement range.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
No claim was made for traceability to a reference standard.
- d. *Detection limit:*
Not applicable.
- e. *Analytical specificity:*
Cross-reactivity: The cross-reactivity was determined using serum samples from patients with Behcet's disease (10), Crohn's disease (10), and Ulcerative colitis (10). The manufacturer states that no cross-reactivity was observed.
Interference: Normal and positive sera samples were spiked with fixed concentrations of hemoglobin, triglycerides, and bilirubin. None of the sera components were found to interfere with assay performance.
- f. *Assay cut-off:*
Qualitative evaluation: Titer of 1:10 or greater that the results in a positive reaction are considered positive. This cut-off was determined based on the evaluation of clinically defined donor samples yielding the appropriate clinical sensitivity and specificity meeting the input criteria. Four hundred eighty-three samples, consisting of 90 Celiac disease patient samples, 30 samples from patients with potentially cross-reacting diseases (Behcet's disease, Crohn's disease, and Ulcerative colitis), and 363 normal healthy adult sera were used for validation. None the 393 normal healthy adult and potential cross-reacting disease samples were positive at a 1:10 dilution.

Semi-quantitative evaluation: The endpoint titer is defined as the highest sample dilution factor for which specific fluorescence is identifiable.

2. Comparison studies:

- a. *Method comparison with predicate device:*
Comparison study between the EUROIMMUN device and the predicate device was performed on 58 clinically diagnosed Celiac disease patient sera. Twenty-eight of which consisted of borderline or low-positive samples. In addition, sera from 10 Behcet's disease patients, 10 Crohn's disease patients, 10 Ulcerative colitis patients, and 148 normal healthy individuals were included. The overall agreement between the new device and predicate was 100%. The results are summarized in the comparison table below:

		Predicate IFA		
		(+)	(-)	Total
EUROIMMUN IFA	(+)	90	0	90
	(-)	0	193	193
	Total	90	193	283

Positive percent agreement: 100% (90/90)
Negative percent agreement: 100% (193/193)
Overall agreement: 100% (283/283)

b. Matrix comparison:

Both assays use human serum.

3. Clinical studies:

a. Clinical sensitivity and specificity:

Comparison studies between the EUROIMMUN device and the predicate device were performed on 120 characterized EMA-related disease samples as described in the method comparison study and 393 normal donor samples. Clinical sensitivity, specificity, and overall agreement are summarized in the table below.

		Clinically diagnosed patient samples		
		(+)	(-)	Total
EUROIMMUN IFA	(+)	90	0	90
	(-)	0	393	393
	Total	90	393	483

Clinical sensitivity: 100% (90/90)

Clinical specificity: 100% (393/393)

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

Not applicable.

4. Clinical cut-off:

See Assay cut-off.

5. Expected values/Reference range:

The expected value in the normal population is negative at a 1:10 dilution.

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.