

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

A. 510(k) Number:

k100017

B. Purpose for Submission:

New device

C. Measurand:

Autoantibodies against glutamate receptor (type NMDA)

D. Type of Test:

Semi-quantitative indirect immunofluorescent antibody (IFA) assay

E. Applicant:

EUROIMMUN US Inc.

F Proprietary and Established Names:

EUROIMMUN Anti-Glutamate receptor (type NMDA) IFA

G. Regulatory Information:

1. Regulation section:
21CFR §866.5660 – Multiple autoantibodies immunological test system
2. Classification:
Class II
3. Product code:
OSK; Anti-glutamate receptor (type NMDA) IFA
4. Panel:
Immunology (82)

H. Intended Use:

1. Intended use(s):
The EUROIMMUN Anti-Glutamate receptor (type NMDA) IFA is intended for the qualitative determination of autoantibodies against glutamate receptor (type NMDA) in human serum. It is used as an aid in the diagnosis of anti- glutamate receptor (type NMDA) autoimmune encephalitis in conjunction with other laboratory and clinical findings.
2. Indication(s) for use:
Same as intended use
3. Special conditions for use statement(s):
Prescription use only
4. Special instrument requirements:
Fluorescent microscope equipped with a 488 nm excitation filter; 510 nm colour separator; & 520 nm blocking filter with a 100 W mercury vapour lamp light source or LED bluelight.

I. Device Description:

The EUROIMMUN IFA is an assay for standardized detection of anti-glutamate receptor (type NMDA) antibodies by indirect immunofluorescence. The test kit consists of slides, which contain BIOCHIPS coated with glutamate receptor (type NMDA) transfected cells and non-transfected cells, fluorescein-labelled anti-human IgG (goat), a positive control for anti-glutamate receptor (type NMDA), a negative control, a salt for preparation of PBS, Tween 20, embedding medium,

cover glasses and an instruction booklet. The non-transfected cells are used as a control to simplify differentiation of potential co-existing and non-specific reactivity such as ANA.

J. Substantial Equivalence Information:

1. Predicate device name(s):
EUROIMMUN ANCA IFA Granulocyte BIOCHIP Mosaic™ Test System.
2. Predicate 510(k) number(s):
k083850
3. Comparison with predicate:
The EUROIMMUN ANCA IFA Granulocyte BIOCHIP Mosaic™ Test System was chosen as a device of equivalent method and technology. There is no exact predicate device available for the detection of antibodies against glutamate receptor (type NMDA).

Similarities		
Item	Device	Predicate
	EUROIMMUN anti-Glutamate receptor (type NMDA) IFA	EUROIMMUN ANCA IFA Granulocyte BIOCHIP Mosaic™ Test System
Intended Use	Semi-quantitative detection of antibodies in human serum	Same
Technology	IFA BIOCHIP TITERPLANE technology using multiple substrates	Same
Sample Type	Serum	Same
Cut Off Level	1:10 dilution	Same

Differences		
Item	Device	Predicate
Analyte	Autoantibodies against glutamate receptor (type NMDA)	Autoantibodies to neutrophil cytoplasmic antigens (ANCA).
Antigens	Glutamate receptor (type NMDA) transfected cells and non-transfected cells	Human granulocytes native antigen

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

None.

L. Test Principle:

Patient samples are diluted 1:10 in PBS-Tween. 25 µL of each diluted patient sample are added to each reaction field of the reagent tray. Reactions are started by fitting the BIOCHIP slides containing the substrates (glutamate receptor type NMDA transfected cells and non-transfected cells) into the corresponding recesses of the reagent tray and incubated for 30 minutes at room temperature.

Specific antibodies attach to the antigens. After incubation the BIOCHIP slides are washed with PBS-Tween to remove unbound antibodies. In the meantime, 20 μ L of fluorescein-labeled anti-human IgG are added to each reaction field of a clean reagent tray and the BIOCHIP slides placed into the recesses of the tray. After a 30 minutes incubation at room temperature, the BIOCHIP slides are again washed with PBS-Tween to remove any unbound fluorescein-labeled reagent. 10 μ L of embedding medium are placed for each reaction field on a cover glass and the BIOCHIP slides, with the BIOCHIPS facing downwards, placed onto the prepared cover glass. Fluorescence is read with a fluorescence microscope.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

The analytical performance of the assay was assessed using a fluorescence intensity level scale which measures the intensity of the specific fluorescence expressed as a numeric value. These values can vary from “0” (no specific fluorescence) to “5” (extremely strong specific fluorescence). The evaluation of the fluorescence intensity is performed according to the following table:

Intensity	Evaluation
0	no specific fluorescence
1	positive reaction, very weak specific reaction visible
2	positive reaction, weak specific reaction visible
3	positive reaction, specific reaction well visible
4	positive reaction, strong specific reaction visible
5	positive reaction, very strong specific reaction visible

The samples used in the analytical and clinical studies were coded. Serological results, clinical and patient data were not known to the technician when performing the tests.

a. *Precision/Reproducibility:*

Intra-assay reproducibility was performed by 10-fold repeated measurements of 6 characterized positive and negative serum samples (at fluorescence intensity levels of 0, 1, 2, and 3). The variation in fluorescence intensity was within ± 1 intensity level unit for type NMDA glutamate receptor antigen.

Inter-assay reproducibility was performed by repeated measurements of 6 characterized positive and negative samples (fluorescence intensity levels of 0, 2, and 3) at five different times (2 measurements per run). The variation in fluorescence intensity was within ± 1 intensity level unit type NMDA glutamate receptor antigen.

In a second inter-assay reproducibility study, four samples with anti-glutamate receptor (type NMDA) antibodies near the cut-off (1-2 fluorescence intensity units) were tested at five different times (2 measurements per run). Each result

was read by three different technicians independently. The variation in fluorescence intensity was within ± 1 intensity level unit for type NMDA glutamate receptor antigen.

Lot-to-lot reproducibility was determined by measurement of 3 characterized positive and negative serum samples (fluorescence intensity levels of 0, 3, and 4) with three different kit lots. The variation in fluorescence intensity was within ± 1 intensity level unit for type NMDA glutamate receptor antigen.

In a second lot-to-lot reproducibility study, four samples with anti-glutamate receptor (type NMDA) antibodies near the cut-off (1-2 fluorescence intensity units) were tested (in duplicate) using three different lots. Each result was read by three different technicians independently. The variation in fluorescence intensity was within ± 1 intensity level unit type NMDA glutamate receptor antigen.

- b. *Linearity/assay reportable range:*
Not applicable.
- c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*
There is no recognized standard or reference material for autoantibodies against glutamate receptor (type NMDA).

Kit stability:

Accelerated stability studies were performed by storing three different kit lots at 37°C and -20°C for 14 days. The kits stored at -20°C were used as a reference. The kits were tested using both positive and negative samples. The results showed that the kits were stable for 14 days at 37°C, which translates into 2 years of stability at 4°C (Deshpande SS (1996) *Enzyme Immunoassays from Concept to Product Development*, Chapman and Hall, International Thomson Publishing, New York, page 388).

- d. *Detection limit:*
Not applicable.
- e. *Analytical specificity:*

Interference:

Three different samples (one negative, one weakly positive, and one positive) were spiked with hemoglobin (up to 500 mg/dL), triglycerides (up to 2000 mg/dL), and bilirubin (up to 40 mg/dL). The deviation in fluorescence intensity due to these spiked samples did not exceed ± 1 fluorescence intensity level unit for rat hippocampus, rat cerebellum, and type NMDA glutamate receptor antigens.

Cross-reactivity:

To investigate the extent of cross-reactivity of the anti-glutamate receptor (type NMDA) assay kit, the following patient samples were tested: 9 autoimmune encephalitis, 15 infectious encephalitis, 3 anti-VGKC autoimmune encephalitis, 2 anti-Ma2 autoimmune encephalitis, and one each anti-GluR2, anti-CV2 autoimmune encephalitis, anti-zic4 cerebellar

degeneration, and anti-recoverin retinopathy. None of these samples exhibited characteristic reactivity against anti-glutamate receptor (type NMDA). Some nonspecific fluorescent staining against rat cerebellum and hippocampus was observed with anti-VGKC, anti-GluR2, and anti-zic4 samples.

f. Assay cut-off:

Cut-off was determined using 17 clinically characterized serum samples from patients with anti-glutamate receptor (type NMDA) autoimmune encephalitis. All samples were found positive at a titer of 1:10 or at higher dilutions. 200 blood samples from healthy blood donors were all found to be negative.

2. Comparison studies:

a. Method comparison with predicate device:

Not applicable.

b. Matrix comparison:

Not applicable.

3. Clinical studies:

a. Clinical Sensitivity and Specificity:

The diagnostic criteria for anti-glutamate receptor (type NMDA) encephalitis included encephalitic signs with psychiatric symptoms (agitation, paranoid thoughts, irritability, or hallucinations), seizures, CSF inflammation, and exclusion of viral/bacterial etiology.

Study 1: 47 serum samples from the US from patients diagnosed with anti-glutamate receptor (type NMDA) encephalitis and controls with other encephalopathies, including anti-VGKC and AMPA receptor encephalitis, were examined. The panel comprised samples from 7 men and 40 women with an average age of 17 years (age range: 5 to 42 years; 1 unknown). In addition, sera of 100 adult healthy blood donors of mixed age and sex from Germany were analyzed. All samples from patients with anti-glutamate receptor (type NMDA) encephalitis (29 sera) were tested positive with the transfected cells, while all disease control samples (18 sera) and healthy blood donors (100 sera) were negative.

Study 2: In a retrospective study, 2990 patients were screened for clinical symptoms of encephalitis of unknown origin. 5 of 6 samples fulfilling the criteria (6 women with an average age of 23 years, age range: 18 to 31 years; origin: Germany) were found positive for antibodies against glutamate receptor (type NMDA). The results support the fact that anti-glutamate receptor (type NMDA) encephalitis is a very frequent cause among these patients.

Study 3: 8 samples from patients with anti-glutamate receptor (type NMDA) encephalitis (origin: Germany) were investigated. The panel comprised samples from 3 men and 5 women with an average age of 25 years (age range: 16 to 39 years). All samples were found positive for anti-glutamate receptor (type NMDA).

Study 4: 9 samples from patients with anti-glutamate receptor (type NMDA) encephalitis and 13 samples from patients with other encephalopathies (origin: Italy) were investigated. The panel comprised samples from 8 men and 14 women with an average age of 47 years (age range: 9 to 89 years). All

samples from patients with anti-glutamate receptor (type NMDA) encephalitis (9 sera) were tested positive with the transfected cells, while all disease control samples (13 sera) were negative.

Panel		n	EUROIMMUN Anti-Glutamate receptor (type NMDA) IFA		
			positive	% positive	95% C.I.
Study 1	Anti-glutamate receptor (type NMDA) encephalitis	29	29	100.0%	88.1 – 100.0%
	Other encephalopathies	18	0	0.0%	0.0 – 18.5%
	Healthy blood donors	100	0	0.0%	0.0 – 3.6%
Study 2	Encephalitis of unknown origin	6	5	83.3%	35.9 – 99.6%
Study 3	Anti-glutamate receptor (type NMDA) encephalitis	8	8	100.0%	63.1 – 100.0%
Study 4	Anti-glutamate receptor (type NMDA) encephalitis	9	9	100.0%	66.4 – 100.0%
	Other encephalopathies	13	0	0.0%	0.0 – 24.7%
Overall sensitivity		52	51 positive	98.1%	89.7 – 100.0%
Overall specificity		131	131 negative	100.0%	97.2 – 100.0%

For these selected populations, sensitivity of the test was 98.1% and specificity was 100% for the disorder. It is expected that sensitivity and specificity would be lower in a screening population.

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

4. Clinical cut-off:

See assay cutoff

5. Expected values/Reference range:

The reference range in the normal population is negative at a 1/10 dilution of serum.

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.