DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
10903 New Hampshire Avenue
Document Control Center – WO66-G609
Silver Spring, MD 20993-0002

August 10, 2016

EURIIMMUN US Inc.
Michael Locke
Director of Regulatory Affairs
1100 The American Road
Morris Plains
NJ 07950

Re: K153303
Trade/Device Name: EUROIMMUN Anti-West Nile Virus ELISA (IgG)
Regulation Number: 21 CFR 866.3940
Regulation Name: West Nile Virus Serological Reagents
Regulatory Class: II
Product Code: NPO
Dated: July 8, 2016
Received: July 12, 2016

Dear Mr. Locke:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration. Please note: CDRH does not evaluate information related to contract liability warranties. We remind you, however, that device labeling must be truthful and not misleading.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to additional controls. Existing major regulations affecting your device can be found in the Code of Federal Regulations, Title 21, Parts 800 to 898. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); medical device reporting (reporting of medical device-related adverse events) (21 CFR 803); good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820); and if applicable, the

electronic product radiation control provisions (Sections 531-542 of the Act); 21 CFR 1000-1050.

If you desire specific advice for your device on our labeling regulations (21 CFR Parts 801 and 809), please contact the Division of Industry and Consumer Education at its toll-free number (800) 638 2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21 CFR Part 807.97). For questions regarding the reporting of adverse events under the MDR regulation (21 CFR Part 803), please go to http://www.fda.gov/MedicalDevices/Safety/ReportaProblem/default.htm for the CDRH's Office of Surveillance and Biometrics/Division of Postmarket Surveillance.

You may obtain other general information on your responsibilities under the Act from the Division of Industry and Consumer Education at its toll-free number (800) 638-2041 or (301) 796-7100 or at its Internet address http://www.fda.gov/MedicalDevices/ResourcesforYou/Industry/default.htm.

Sincerely yours,

Steven R. Gitterman -S

for Uwe Scherf, M.Sc., Ph.D.
Director
Division of Microbiology Devices
Office of *In Vitro* Diagnostics and
Radiological Health
Center for Devices and Radiological Health

Enclosure

DEPARTMENT OF HEALTH AND HUMAN SERVICES
Food and Drug Administration

Indications for Use

Form Approved: OMB No. 0910-0120
Expiration Date: January 31, 2017
See PRA Statement on last page.

510(k) Number *(if known)*
K153303

Device Name
EUROIMMUN Anti-West Nile Virus ELISA (IgG)

Indications for Use *(Describe)*
The EUROIMMUN Anti-West Nile Virus ELISA (IgG) is intended for the qualitative detection of IgG antibodies to West Nile virus in human serum and plasma (K+-EDTA, Li+-heparin). This test is intended as an aid in the presumptive laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with meningitis/encephalitis, in conjunction with other laboratory and clinical findings. Positive results must be confirmed by the plaque reduction neutralization test (PRNT) or by using the current CDC guidelines for diagnosis of this disease.

The assay characteristics have not been established for testing cord blood, neonates, prenatal screening, and general population screening of patients without symptoms of meningoencephalitis. This assay is not FDA cleared or approved for testing blood or plasma donors.

Warning: Cross-reactivity with IgG to Dengue, Chikungunya, Zika and Tick-borne Encephalitis (TBE) viruses has been observed with the EUROIMMUN Anti-West Nile Virus ELISA (IgG). Reactive results must be reported with a caution statement regarding possible IgG cross-reactivity with other flaviviruses.

Type of Use *(Select one or both, as applicable)*

☒ Prescription Use (Part 21 CFR 801 Subpart D) ☐ Over-The-Counter Use (21 CFR 801 Subpart C)

PLEASE DO NOT WRITE BELOW THIS LINE – CONTINUE ON A SEPARATE PAGE IF NEEDED.

FOR FDA USE ONLY

Concurrence of Center for Devices and Radiological Health (CDRH) *(Signature)*

This section applies only to requirements of the Paperwork Reduction Act of 1995.

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510(k) SUBSTANTIAL EQUIVALENCE

510(k) Number

K153303

Applicant

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Contact: Michael Locke
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Fax: 973-656-1098

Product Name

EUROIMMUN Anti-West Nile Virus ELISA (IgG)

Device Identification

Regulation: 21 CFR 866.3940 (West Nile virus serological reagents)
Classification: Class II
Product Code: NOP
Panel: Microbiology

Substantial Equivalence Information:

Comparison to Predicate Device

Similarities

Item	**New Device** EUROIMMUN Anti-West Nile Virus ELISA (IgG) (K153303)	**Predicate Device** Focus Diagnostics West Nile Virus IgG DxSelect™ (K031953)
Intended use	Detection of IgG class antibodies against West Nile virus	Same
Assay format	Qualitative	Same
Technology	ELISA	Same
Assay platform	96-well microtiter plates	Same
Antigen	Coated on microtiter plate	Same
Calibrators and Controls	1 calibrator (cut-off) 2 controls: 1 positive; 1 negative	Same
Conjugate	Anti-human IgG (rabbit) labelled with horseradish peroxidase	Same
Substrate	TMB	Same
Wash buffer	10x concentrate	Same
Serum sample dilution	1:101	Same
Procedure	Sample incubation with micro-well antigen coated plate, followed by a wash step, incubation with an anti-human IgG enzyme conjugate; wash step, incubation with substrate; stopping of the reaction with stop solution, photometric reading.	Same

Differences

Item	**New Device**	**Predicate Device**
Antigen	Recombinant, detergent-extracted	Recombinant West Nile virus

	glycoprotein E of West Nile virus from the membrane fraction of human cells, inactivated using high temperatures and gamma radiation; effectiveness of inactivation tested by culture		antigen	
Stop solution	0.5 M sulphuric acid		1 M sulfuric acid	
Reagent preparation	All reagents, calibrator and controls are ready to use, except for the wash buffer.		Calibrator and controls require dilution before use.	
Sample matrix	Serum or plasma (EDTA, Li-heparin), CSF		Serum	
Reported results	Ratio		Index	
Cut-off levels	Ratio	Result	Index	Result
	<0.8	negative	< 1.30	negative
	≥0.8 to <1.1	borderline	≥ 1.30 to < 1.50	equivocal
	≥1.1	positive	≥ 1.50	positive

Intended Use

The EUROIMMUN Anti-West Nile Virus ELISA (IgM) is intended for the qualitative detection of IgM antibodies to West Nile virus in human serum and plasma (K^+-EDTA, Li^+-heparin). This test is intended as an aid in the presumptive laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with meningitis/encephalitis, in conjunction with other laboratory and clinical findings. Positive results must be confirmed by the plaque reduction neutralization test (PRNT) or by using the current CDC guidelines for diagnosis of this disease.

The assay characteristics have not been established for testing cord blood, neonates, prenatal screening, and general population screening of patients without symptoms of meningoencephalitis. This assay is not FDA cleared or approved for testing blood or plasma donors.

Warning: Cross-reactivity with IgG from Dengue, Chikungunya, Zika and Tick-borne Encephalitis (TBE) viruses has been observed with the EUROIMMUN Anti-West Nile Virus ELISA (IgG). Reactive results must be reported with a caution statement regarding possible IgG cross-reactivity with other flaviviruses.

Device Description

Patient samples are diluted 1:101 in sample buffer, 100 µl of each diluted patient sample and pre-diluted controls and the calibrator are added to the antigen coated microtiter wells and incubated for 60 minutes at +37°C. After incubation the microtiter well strips are washed 3 times with wash buffer to remove unbound antibodies and 100 µl of the anti-human IgG enzyme conjugate reagent is added to each microtiter well. After an additional 30-minutes incubation at room temperature, the microtiter wells are again washed 3 times with wash buffer to remove any unbound enzyme conjugate and 100 µl of the chromogen substrate is added. The strips are incubated for 15 minutes at room temperature and 100 µl stop solution is added. The microtiter plates are placed in an ELISA reader and read at a wavelength of 450 nm and a reference wavelength of between 620 nm and 650 nm within 30 minutes.

Test Principle

The test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigens. In the first reaction step, diluted patient samples, calibrator and controls are incubated in the wells. Anti-West Nile virus antibodies will bind to the antigens coated in the microtiter wells. The wells are washed to remove any unbound proteins and non-specific antibodies. In a second reaction step, rabbit anti-human IgG HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have human IgG binding to the West Nile virus antigen. The wells are washed to remove any unbound HRP enzyme conjugate. 3,3,5,5 tetramethylbenzidine (TMB) enzyme substrate is added. If the HRP enzyme is present in the well (positive reaction), the HRP enzyme will react with the TMB substrate and produce a blue color. After an additional incubation time to allow the color development, a stop solution is added which turns the blue color yellow and inhibits further color development to allow for a stable spectrophotometric reading. The test strips are placed in a microplate reader and the optical density of the color is measured. The amount of antigen specific bound antibody is proportional to the color intensity.

Performance Characteristics

Analytical Performance

Repeatability/Reproducibility

Repeatability: The repeatability of the EUROIMMUN Anti-West Nile Virus ELISA (IgG) was investigated by testing of a panel of 7 members prepared using native/natural patient samples seropositive at different concentrations. The inter-assay repeatability is based on 42 determinations per sample performed in 14 different runs on 7 different days (with 2 runs per day and 3 replicates per run). The data from the repeatability study is presented in the table below.

Repeatability

No.	Mean Ratio	Within-Run		Within Day		Between Days		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.1	0.01	7.4%	0.01	13.2%	0.00	1.3%	0.01	13.3%
2	0.4	0.02	5.3%	0.04	11.7%	0.03	6.9%	0.05	13.6%
3	0.8	0.03	4.2%	0.07	9.7%	0.00	0.0%	0.07	9.7%
4	0.9	0.03	3.7%	0.09	10.4%	0.00	0.0%	0.09	10.4%
5	1.2	0.05	4.2%	0.08	6.7%	0.00	0.0%	0.08	6.7%
6	2.2	0.08	3.8%	0.14	6.1%	0.00	0.0%	0.14	6.1%
7	3.7	0.06	1.7%	0.20	5.5%	0.13	3.6%	0.24	6.6%
8	4.1	0.14	3.5%	0.23	5.7%	0.09	2.3%	0.25	6.1%

Reproducibility: The reproducibility of the EUROIMMUN Anti-West Nile Virus ELISA (IgG) was investigated by testing of a panel of 7 members prepared using native/natural patient samples seropositive at different concentrations. The reproducibility is based on 60 determinations per sample performed at 3 different sites (in-house; and 2 laboratories in the north-east) for 5 days with 2 runs per day and 2 replicates per run. The data from the reproducibility study is presented in the table below.

Reproducibility

No.	Mean Ratio	Within-Run		Within Day		Between Days		Between Sites		Total	
		SD	%CV	SD	%CV	SD	%CV	SD	%CV	SD	%CV
1	0.1	0.010	14.1%	0.011	15.0%	0.005	6.4%	0.004	5.3%	0.012	16.3%
2	0.7	0.072	11.0%	0.096	14.7%	0.000	0.0%	0.063	9.7%	0.096	14.7%
3	0.8	0.062	8.0%	0.075	9.8%	0.017	2.2%	0.043	5.7%	0.077	10.1%
4	0.8	0.070	8.3%	0.098	11.6%	0.000	0.0%	0.069	8.1%	0.098	11.6%
5	1.1	0.089	7.8%	0.119	10.5%	0.000	0.0%	0.079	7.0%	0.119	10.5%
6	2.2	0.211	9.7%	0.211	9.7%	0.060	2.7%	0.010	0.5%	0.219	10.1%
7	4.3	0.405	9.4%	0.436	10.1%	0.000	0.0%	0.161	3.7%	0.436	10.1%

Analytical Specificity/Cross Reactivity

Cross Reactivity: Cross Reactivity was investigated using 910 serologically characterized seropositive specimens from patients with diseases other than WNV. Each of the specimens included in the study was characterized with respect to disease state prior to analysis of the specimens with the EUROIMMUN Anti-West Nile Virus ELISA (IgG). Cross-reactivity across the flavivirus group is common (i.e., St. Louis encephalitis, Dengue 1, 2, 3 & 4; Murray Valley encephalitis, Japanese encephalitis, Yellow fever viruses and Zika virus); as well as persons vaccinated for flaviviruses.

Cross Reactivity

No.	Panel	n	Anti-West Nile Virus ELISA (IgG)		
			Positive	Negative	% Negative
1	Anti-Adenovirus	12	0	12	100.0%
2	Anti-Barmah Forest virus	20	0	20	100.0%
3	Anti-Borrelia burgdorferi	54	3	51	94.4%
4	Anti-Chikungunya virus	72	23	49	68.1%
5	Anti-Chlamydia pneum.	12	0	12	100.0%
6	Anti-CMV	12	0	12	100.0%

No.	Panel	n	Anti-West Nile Virus ELISA (IgG)		
			Positive	Negative	% Negative
7	Anti-Dengue virus	58	50	8	13.8%
8	Anti-EBV	60	2	58	96.7%
9	Anti-Hanta virus	11	0	11	100.0%
10	Anti-Hepatitis virus	32	0	32	100.0%
11	Anti-Helicobacter pylori	12	0	12	100.0%
12	Anti-HSV-1	39	2	37	94.9%
13	Anti-Influenza A	12	0	12	100.0%
14	Anti-Influenza B	12	0	12	100.0%
15	Anti-Leptospira	11	0	11	100.0%
16	Malaria/anti-Plasmodium falciparum	8	0	8	100.0%
17	Anti-Measles virus	12	0	12	100.0%
18	Anti-Mumps virus	12	0	12	100.0%
19	Anti-Mycoplasma pneumoniae	12	0	12	100.0%
20	Anti-Parainfluenza types 1-4	12	0	12	100.0%
21	Anti-Polio virus	37	1	36	97.3%
22	Anti-Ross River virus	20	1	19	95.0%
23	Anti-RSV	12	0	12	100.0%
24	Anti-Rubella virus	12	0	12	100.0%
25	Anti-TBE virus	118	33	85	72.0%
26	Anti-Toxoplasma gondii	9	0	9	100.0%
27	Anti-VZV	32	0	32	100.0%
28	Anti-West Nile Virus IgM	10	0	10	100.0%
29	Yellowfever virus immunization	12	0	12	100.0%
30	Anti-Zika virus	47	47	0	0.0%
31	Rheumatoid arthritis/polyarthritis/anti-CCP	16	0	16	100.0%
32	Anti-Rheumatoid factor	37	1	36	97.3%
33	Anti-nuclear autoantibodies	33	1	32	97.0%
34	ANCA-associated small vessel vasculitides/ANCA	6	0	6	100.0%
35	Celiac disease/anti-endomysium	10	0	10	100.0%
36	Plasma cell myeloma	14	0	14	100.0%
	Total	910	164	746	82.0%

Interferences: Hemolytic, lipemic and icteric samples showed no influence on the result up to a concentration of 1000 mg/dl for hemoglobin, 2000 mg/dl for triglycerides and 40 mg/dl for bilirubin in testing with the EUROIMMUN Anti-West Nile Virus ELISA (IgG). Interferences to high protein (albumin), cholesterol, and intralipids were not investigated.

Assay Cut-off:

The recommended assay cut-off is based on OD results of 18 sera from clinically characterized positive West Nile virus patients, and of 150 sera from normal healthy blood donors from a non-endemic region (100 men, 50 women; mean age 39.9 years, age range 18 to 68 years). The samples were investigated using the EUROIMMUN Anti-West Nile Virus ELISA (IgG) and a ROC analysis was performed using the OD's obtained. The ROC analysis demonstrated optimal sensitivity (100.0%) and specificity (100.0%) at the OD value of 0.475. The calibrator was established at this cut-off OD.

The borderline range of ratio 0.8 to ratio 1.1 was established to cover at least 98% of the negative samples (148 of 150 samples) in the negative range.

Using the cut-off of ratio 1.0 and borderline range of ratio 0.8 to 1.1 with the positive and negative groups mentioned above, the EUROIMMUN Anti-West Nile Virus ELISA (IgG) showed a sensitivity of 100.0% (95% C.I.: 81.5 – 100.0%) with a specificity of 98.7% (95% C.I.: 95.3 – 99.8%; if borderline samples counted as positive).

Clinical Study I:

A prospective clinical study was performed with 152 samples from patients suspected of West Nile Virus infection collected at hospitals and clinics across the US in 2015. The panel consisted of 81 men and 71 women, age ranged from 6 to 85 years with a mean age of 49 years. Each specimen was tested at one internal and two external sites with the EUROIMMUN Anti-West Nile Virus ELISA (IgG) in parallel with the predicate assay. Average of three results for each clinical specimen tested at three sites was considered to calculate the positive percent agreement and negative percent agreement between the EUROIMMUN Anti-West Nile Virus ELISA (IgG) vs the predicate assay. The following results were obtained.

Serum n = 152		**Predicate**		
		Positive	Borderline	Negative
EUROIMMUN Anti- West Nile Virus ELISA (IgG)	Positive	42	1	1
	Borderline	2	0	4
	Negative	1	0	101

Positive agreement	93.3% (42/45)	**95% C.I.**	81.7-98.6%
Negative agreement	94.4% (101/107)	**95% C.I.**	88.2-97.9%

Of the 45 presumptive positives by the predicate device, 27 samples were further tested by PRNT (Plaque Reduction Neutralization Test) and the EUROIMMUN Anti-West Nile Virus ELISA (IgG). The results are shown below.

Serum n = 27		**PRNT Results**		
		Positive	Borderline	Negative
EUROIMMUN Anti- West Nile Virus ELISA (IgG)	Positive	25	0	0
	Borderline	1	0	0
	Negative	1	0	0

Sensitivity	92.6% (25/27)	**95% C.I.**	75.7-99.1%

Clinical Study II:

A study was performed at a clinical laboratory in the midwest, with 401 serum samples collected prospectively from patients suspected of west nile virus infection. The serum panel consisted of 195 men and 206 women, age ranged from 3 to 102 years with a mean age of 47 years. The samples were tested with the EUROIMMUN Anti-West Nile Virus ELISA (IgG) in parallel with the Predicate ELISA.

Serum n = 152		**Predicate**		
		Positive	Borderline	Negative
EUROIMMUN Anti- West Nile Virus ELISA (IgG)	Positive	43	0	3
	Borderline	1	0	1
	Negative	1	0	352

Positive agreement	95.6% (43/45)	**95% C.I.**	84.9-99.5%
Negative agreement	98.9% (352/356)	**95% C.I.**	97.1-99.7%

Clinical Study III:

A retrospective clinical study was performed in cooperation with the Robert Koch Institute (RKI), Berlin, Germany with 295 serum samples that included 200 samples from major outbreaks of West Nile fever in South Africa in 1974 and 1984. The samples were tested by the PRNT and the EUROIMMUN Anti-West Nile Virus ELISA (IgG). The results are shown below.

Serum n = 295		**RKI Anti-West Nile Virus PRNT**	
		Positive	Negative
EUROIMMUN Anti- West Nile Virus ELISA (IgG)	Positive	194	3
	Borderline	0	0
	Negative	1	97

Positive agreement	99.5% (194/195)	**95% C.I.**	97.2-100.0%
Negative agreement	97.0% (97/100)	**95% C.I.**	91.5-99.4%

Expected Values

Euroimmun assessed reactivity with 553 samples prospectively collected from US population. The samples consisted of 50% females, and 50% males The range of positivity of different populations from the US prospective studies with the EUROIMMUN Anti-West Nile Virus ELISA (IgG) test kit are presented below.

US Studies

Age	n	Negative	Borderline	Positive	% Positive	95% C.I.
0-9	16	16	0	0		0.0 – 20.6%
10-19	32	30	0	2	6.3% (2/32)	0.8 – 20.8%
20-29	66	59	2	5	7.6% (5/66)	2.5 – 16.8%
30-39	86	74	1	11	12.8% (11/86)	6.6 – 21.7%
40-49	89	76	1	12	13.5% (12/89)	7.2 – 22.4%
50-59	100	79	2	19	19.0% (19/100)	11.8 – 28.1 %
60+	164	121	2	41	25.0% (41/164)	18.6 – 32.3%
total	553	455	8	90	16.3% (90/455)	13.3 – 19.6%

Note: It is recommended that each laboratory determine its own normal range based on the population and equipment used.

Matrix Comparison (Serum vs Plasma)

The usability of plasma was investigated using sample pairs each of serum and corresponding plasma (EDTA, Li-heparin). As no real plasma sample pairs from patients containing anti-West Nile virus antibodies were available, the samples were created from 5 different sets of normal blood donor sample pairs (serum, EDTA plasma, Li-heparin plasma) that were (after drawing) spiked with 5 different positive serum samples. After spiking, the sample sets were further diluted and processed according to the package insert.

Passing-Bablok regression was calculated for the comparison of serum to plasma. The regression equation is near the ideal correlation (intercept 0; slope 1.0) indicating equivalence of concentrations between serum and the corresponding plasma matrices. Coefficients of determination were found to be above 0.975 and % recovery compared to serum was in the range of 92 to 109% (serum = 100%).

Matrix Comparison (serum vs plasma)

	EDTA plasma	Li-heparin plasma
Determinations (n)	20	20
Concentration range (serum)	Ratio 0.6 – 4.1	Ratio 0.6 – 4.1
Concentration range (plasma)	Ratio 0.6 – 4.1	Ratio 0.6 – 4.1
Regression equation (y = plasma, x = serum)	y = -0.01 + 1.02x	y = 0.04 + 0.96x
95% C.I. of intercept	-0.05 – 0.02	-0.11 – 0.15
95% C.I. of slope	0.99 – 1.05	0.89 – 1.10
Coefficient of determination R^2	0.9983	0.9759
Mean %recovery	101 %	100 %
Range of %recovery	98 – 105 %	92 – 109 %

Conclusion

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