

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

A. 510(k) Number:

K153303

B. Purpose for Submission:

Clearance of new device

C. Measurand:

IgG class antibodies to West Nile virus

D. Type of Test:

ELISA for the qualitative detection of IgG class antibodies

E. Applicant:

EUROIMMUN US, INC.

F. Proprietary and Established Names:

EUROIMMUN Anti-West Nile Virus ELISA (IgG)

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3940 – West Nile virus serological reagents

2. Classification:

Class II

3. Product code:

NOP

4. Panel:

Microbiology (83)

H. Intended Use:

1. Intended use(s):

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 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.

2. Indication(s) for use:

Same as Intended Use

3. Special conditions for use statement(s):

Not Applicable

4. Special instrument requirements:

Not Applicable

I. Device Description:

The EUROIMMUN Anti-West Nile Virus ELISA (IgG) test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigen. 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 horseradish peroxidase (HRP) 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.

The antigen source in the EUROIMMUN Anti-West Nile Virus ELISA (IgG) is a recombinant, detergent-extracted glycoprotein E of West Nile virus from the membrane fraction of human cells

J. Substantial Equivalence Information:

1. Predicate device name(s):

Focus Diagnostics West Nile Virus IgG DxSelect™

2. Predicate 510(k) number(s):

K031953

3. Comparison with predicate:

Similarities

Item	New Device EUROIMMUN Anti-West Nile Virus ELISA (IgG) (K153303)	Predicate Device Focus Diagnostics West Nile Virus IgG DxSelect™ ELISA (K031953)
Intended use	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 meningitis/encephalitis, in conjunction with other laboratory and clinical findings. Positive results must be confirmed by plaque reduction neutralization test (PRNT) or by using the current CDC guidelines for diagnosis of this disease.	The Focus Diagnostics West Nile Virus IgG DxSelect™ is intended for qualitatively detecting IgG antibodies to West Nile virus in human serum. In conjunction with the Focus Diagnostics West Nile Virus IgM Capture DxSelect™, the test is indicated for testing persons having symptoms of meningioencephalitis, as an aid in the presumptive laboratory diagnosis of West Nile virus infection. Positive results must be confirmed by neutralization test, or by using the current CDC guidelines for diagnosing West Nile encephalitis. This test is not intended for self-testing, and this test is not FDA cleared nor approved for testing blood or plasma donors. Assay performance characteristics have not been established for automated instruments.
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 EUROIMMUN Anti-West Nile Virus ELISA (IgG) (K153303)	Predicate Device Focus Diagnostics West Nile Virus IgG DxSelect™ ELISA (K031953)																
Antigen	Recombinant, detergent-extracted 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	Recombinant West Nile virus 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),	Serum																
Reported results	Ratio	Index																
Cut-off levels	<table border="0"> <tr> <td><u>Ratio</u></td> <td><u>Result</u></td> </tr> <tr> <td><0.8</td> <td>negative</td> </tr> <tr> <td>≥0.8 to <1.1</td> <td>borderline</td> </tr> <tr> <td>≥1.1</td> <td>positive</td> </tr> </table>	<u>Ratio</u>	<u>Result</u>	<0.8	negative	≥0.8 to <1.1	borderline	≥1.1	positive	<table border="0"> <tr> <td><u>Index</u></td> <td><u>Result</u></td> </tr> <tr> <td>< 1.30</td> <td>negative</td> </tr> <tr> <td>≥ 1.30 to < 1.50</td> <td>equivocal</td> </tr> <tr> <td>≥ 1.50</td> <td>positive</td> </tr> </table>	<u>Index</u>	<u>Result</u>	< 1.30	negative	≥ 1.30 to < 1.50	equivocal	≥ 1.50	positive
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K. Standard/Guidance Document Referenced (if applicable):

Class II Special Controls Guidance Document: Serological Reagents for the Laboratory

L. Test Principle:

In the first reaction step, diluted patient samples, calibrators 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 bound to the West Nile virus antigen. The wells are washed to remove any unbound HRP enzyme conjugate. Enzyme substrate (3,3',5,5' tetramethylbenzidine (TMB)) is then added. If the HRP enzyme is present in the well, the HRP enzyme will react with the TMB substrate and produce a blue color indicating a positive result. After an additional incubation time to allow the color development, a stop solution is added which turns the color from blue to 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/ Repeatability:

The repeatability of the EUROIMMUN Anti-West Nile Virus ELISA (IgG) was investigated by testing of a panel of 7 members prepared using natural patient samples seropositive at different levels of antibody. 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.

Precision/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%

b. Reproducibility:

The reproducibility of the EUROIMMUN Anti-West Nile Virus ELISA (IgG) was

investigated by testing of a panel of 7 members prepared using natural patient samples seropositive at different levels of antibody. The reproducibility is based on 60 determinations per sample performed at 3 different sites (in-house, and 2 external laboratories) 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%

c. *Linearity/assay reportable range:*

Not applicable

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

Not applicable

e. *Detection limit:*

Not applicable

f. *Analytical specificity/Cross-reactivity:*

Cross-reactivity was investigated using 910 serologically characterized seropositive specimens from patients with diseases other than West Nile virus. 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 (e.g., St. Louis encephalitis, Dengue 1, 2, 3 & 4; Murray Valley encephalitis, Japanese encephalitis, and Yellow fever viruses) 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%
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	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%

g. Interference:

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 from high protein (albumin), cholesterol, and intralipids were not investigated.

h. Assay cut-off:

The assay cut-off and borderline range was established based on a ROC analysis of 18 sera from clinically characterized positive West Nile virus patients and 150 sera from normal healthy individuals from a non-endemic region.

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 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).

2. Comparison studies:

a. Method comparison with predicate device:

The EUROIMMUN Anti-West Nile Virus ELISA (IgG) was compared with two reference assays: The predicate device Focus Diagnostics West Nile Virus IgG Capture DxSelect™ (K153303) and the plaque reduction neutralization test (PRNT).

b. Matrix comparison:

Serum vs plasma comparison: The usability of plasma was investigated using serum paired with the corresponding plasma (EDTA, Li-heparin). Passing-Bablok regression was calculated for the comparison of serum to plasma. The regression equations indicated equivalence of 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%).

Serum vs Plasma Comparison

	EDTA plasma	Li-heparin plasma
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 R²	0.9983	0.9759
Mean % recovery	101 %	100 %
Range of % recovery	98 - 105 %	92 - 109 %

3. Clinical studies:

a. *Clinical Sensitivity:*

Not Applicable

b. *Clinical specificity:*

Not Applicable

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

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 year 2015. The panel consisted of 81 men and 71 women; the ages 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. The 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.

Clinical Study I: EUROIMMUN Anti-West Nile Virus ELISA (IgG) against predicate

Serum n = 152		Predicate Assay		
		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	94.39% (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 and the EUROIMMUN Anti-West Nile Virus ELISA (IgG). The results are shown below.

Clinical Study I: EUROIMMUN Anti-West Nile Virus ELISA (IgG) against PRNT

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%
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Clinical Study II:

A study was performed at a clinical laboratory in the midwest, with 401 serum samples collected prospectively from patients suspected of infection with West Nile virus. The patients consisted of 195 men and 206 women; the ages 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.

Clinical Study II: EUROIMMUN Anti-West Nile Virus ELISA (IgG) against predicate

Serum n = 401		Predicate Assay		
		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%
C 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 PRNT and the EUROIMMUN Anti-West Nile Virus ELISA (IgG). The results are shown below.

Clinical Study II: EUROIMMUN Anti-West Nile Virus ELISA (IgG) against PRNT

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

Sensitivity	99.5% (194/195)	95% C.I.	97.29-100.0%
Specificity	97.0% (97/100)	95% C.I.	91.5-99.4%

4. Clinical cut-off:

Not Applicable

5. Expected values:

Euroimmun assessed reactivity with 553 samples prospectively collected from patients in the US. 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.

Expected Values from 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%

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.