

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

**A. 510(k) Number:**

K153308

**B. Purpose for Submission:**

Clearance of new device

**C. Measurand:**

IgM class antibodies to West Nile virus

**D. Type of Test:**

ELISA for the qualitative detection of IgM class antibodies

**E. Applicant:**

EUROIMMUN US, INC.

**F. Proprietary and Established Names:**

EUROIMMUN Anti-West Nile Virus ELISA (IgM)

**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 (IgM) is intended for the qualitative detection of IgG antibodies to West Nile virus in human serum and plasma (K<sup>+</sup>-EDTA, Li<sup>+</sup>-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 IgM to Dengue virus, Malaria/anti-Plasmodium falciparum, and Parovirus B19 has been observed with the EUROIMMUN Anti-West Nile Virus ELISA (IgM). 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 (IgM) test kit contains 12 microtiter strips each with 8 break-off reagent wells coated with West Nile virus antigen. Patient serum or plasma samples are diluted 1:101 in sample buffer and incubated for 10 minutes at room temperature to allow IgG/RF separation. 100 µl of each diluted patient sample and pre-diluted controls and 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 IgM 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 (IgM) 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 IgM DxSelect™ ELISA

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

K040854

3. Comparison with predicate:

**Similarities**

<b>Item</b>	<b>New Device</b> EUROIMMUN Anti-West Nile Virus ELISA (IgM) (K153308)	<b>Predicate Device</b> Focus Diagnostics West Nile Virus IgM Capture DxSelect™ ELISA (K040854)
Intended use	The EUROIMMUN Anti-West Nile Virus ELISA (IgM) is intended for the qualitative detection of IgG antibodies to West Nile virus in human serum and plasma (K <sup>+</sup> -EDTA, Li <sup>+</sup> -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 plaque reduction neutralization test (PRNT) or by using the current CDC guidelines for diagnosis of this disease.	The Focus Diagnostics West Nile Virus IgM Capture DxSelect™ is intended for qualitatively detecting IgM antibodies to West Nile virus in human serum. In conjunction with the Focus Diagnostics West Nile Virus IgG Capture DxSelect™, the test is indicated for testing persons having symptoms of meningoencephalitis, as an aid in the presumptive laboratory diagnosis of West Nile virus infection. Positive results must be tested using the background subtraction method (either on the initial test or on a repeat test). 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 IgM enzyme conjugate; wash step, incubation with substrate; stopping of the reaction with stop solution, photometric reading.	Same
<b>Differences</b>		
<b>Item</b>	<b>New Device</b> EUROIMMUN Anti-West Nile Virus ELISA (IgM) (K153308)	<b>Predicate Device</b> Focus Diagnostics West Nile Virus IgM DxSelect™ ELISA (K040854)
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
Procedure	IgG/RF removal by incubation with sample buffer containing IgG/RF-Absorbent.	Capture-Technology: Samples react with wells coated with anti-human IgM; antigen specific for West Nile

			virus added in an additional incubation/wash step.	
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

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

Class II Special Controls Guidance Document: Serological Reagents for the Laboratory Diagnosis of West Nile Virus, October 30, 2003.

**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 IgM HRP enzyme conjugate is added to each well. The enzyme conjugate will bind to any wells that have human IgM 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 (IgM) was investigated by testing of a panel of 8 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	6.9%	0.01	10.5%	0.0	3.3%	0.02	13.0%
2	0.4	0.02	5.2%	0.02	5.2%	0.05	13.1%	0.06	14.1%
3	0.9	0.05	5.2%	0.06	7.2%	0.03	3.8%	0.07	8.2%
4	1.0	0.03	2.8%	0.05	5.5%	0.05	5.4%	0.07	7.7%
5	1.1	0.04	3.8%	0.09	8.0%	0.10	8.9%	0.13	11.9%
6	2.4	0.05	2.3%	0.20	8.6%	0.00	0.0%	0.20	8.6%
7	3.9	0.06	1.5%	0.28	7.3%	0.00	0.0%	0.28	7.3%
8	4.9	0.08	1.7%	0.33	6.8%	0.00	0.0%	0.33	6.8%

b. *Reproducibility:*

The reproducibility of the EUROIMMUN Anti-West Nile Virus ELISA (IgM) 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	0.0	0.01	10.3%	0.003	5.2%	0.00	0.0%	0.00	3.9%	0.01	15.1%
2	0.7	0.08	11.7%	0.000	0.0%	0.00	0.0%	0.04	5.9%	0.09	14.1%
3	0.9	0.06	6.7%	0.046	5.1%	0.03	3.0%	0.00	0.0%	0.09	10.2%
4	1.2	0.11	9.2%	0.044	3.6%	0.00	0.0%	0.00	0.0%	0.14	11.8%
5	1.3	0.06	4.9%	0.048	3.8%	0.00	0.0%	0.06	4.8%	0.13	9.9%
6	2.3	0.11	4.6%	0.070	3.1%	0.00	0.0%	0.10	4.3%	0.20	9.0%
7	4.9	0.26	5.3%	0.223	4.6%	0.15	2.9%	0.00	0.0%	0.42	8.5%

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 692 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 (IgM). Cross-reactivity across the flavivirus group is common (e.g., St. Louis encephalitis, Dengue 1, 2, 3 & 4; Murray Valley encephalitis, Japanese encephalitis, Yellow fever viruses, and Zika virus).

Cross-Reactivity

No.	Panel	n	Anti-West Nile Virus ELISA (IgM)		
			Positive	Negative	% Negative
1	Anti-Barmah Forest virus	20	0	20	100.0%
2	Anti-Borrelia burgdorferi	50	0	50	100.0%
3	Anti-Chikungunya virus	59	1	58	98.3%
4	Anti-CMV	16	0	16	100.0%
5	Anti-Dengue virus	57	14	43	75.4%
6	Anti-EBV	56	2	54	96.4%
7	Anti-Hanta virus	4	0	4	100.0%
8	Anti-Hepatitis virus	12	0	12	100.0%
9	Anti-HSV-1	29	0	29	100.0%
10	Anti-Leptospira	17	0	17	100.0%
11	Malaria/anti-Plasmodium falciparum	10	4	6	60.0%
12	Anti-Measles virus	18	1	17	94.4%
13	Anti-Mumps virus	14	0	14	100.0%
14	Anti-Parvovirus B19	7	2	5	71.4%
15	Anti-Polio virus	21	0	21	100.0%
16	Anti-Ross River virus	20	0	20	100.0%
17	Anti-Rubella virus	10	0	10	100.0%
18	Anti-TBE virus	31	3	28	90.3%
19	Anti-Toxoplasma gondii	13	1	12	92.3%
20	Anti-VZV	32	0	32	100.0%
21	Anti-West Nile Virus	13	0	13	100.0%
22	Yellowfever virus immunization	31	2	29	93.5%
23	Anti-Zika virus	47	2	44	93.6%
24	Rheumatoid arthritis/polyarthritis/anti-CCP	16	0	16	100.0%
25	Anti-Rheumatoid factor	39	0	39	100.0%
26	Anti-nuclear autoantibodies	20	0	20	100.0%
27	ANCA-associated small vessel vasculitides/ANCA	6	0	6	100.0%
28	Celiac disease/anti-endomysium	10	0	10	100.0%
29	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 (IgM). 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 (97.3%) at the OD value of 0.217. 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 95% of the negative samples (143 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 (IgM) showed a sensitivity of 100.0% (95% C.I.: 81.5 – 100.0%) with a specificity of 95.3% (95% C.I.: 90.6 – 98.1%).

2. Comparison studies:

a. *Method comparison with predicate device:*

The EUROIMMUN Anti-West Nile Virus ELISA (IgM) was compared with two reference assays: The predicate device Focus Diagnostics West Nile Virus IgM Capture DxSelect™ ELISA (K040854) and the plaque-reduction neutralization test (PRNT).

b. *Matrix comparison:*

**Serum vs plasma comparison:** The usability of plasma was investigated using sample pairs each of 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 0.970 and % recovery compared to serum was in the range of 83 to 123% (serum = 100%).

Serum vs Plasma Comparison

	<b>EDTA plasma</b>	<b>Li-heparin plasma</b>
<b>n</b>	20	20
<b>Concentration range (serum)</b>	Ratio 0.3 - 2.6	Ratio 0.3 - 2.6
<b>Concentration range (plasma)</b>	Ratio 0.3 - 2.5	Ratio 0.3 - 2.4
<b>Regression equation</b> (y = Plasma, x = Serum)	$y = 0.01 + 1.00x$	$y = 0.02 + 0.92x$
<b>95% C.I. of intercept</b>	-0.05 - 0.07	-0.05 - 0.12
<b>95% C.I. of slope</b>	0.93 - 1.06	0.83 - 0.98
<b>Coefficient of determination R<sup>2</sup></b>	0.9787	0.9707
<b>Mean % recovery</b>	101 %	98 %
<b>Range of % recovery</b>	86 - 116 %	83 - 123 %

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 155 samples from patients suspected of West Nile Virus infection collected at hospitals and clinics across the US in 2015. The panel consisted of samples from 83 men and 72 women; 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 (IgM) 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 (IgM) vs reference assay. The following results were obtained.

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

Serum n = 152		Predicate Assay		
		Positive	Borderline	Negative
<b>EUROIMMUN Anti- West Nile Virus ELISA (IgM)</b>	Positive	36	1	0
	Borderline	4	1	2
	Negative	2	0	109

**Positive Agreement**            85.7% (36/42)        **95% C.I.**    71.5-94.6%  
**Negative Agreement**        97.3% (109/112)    **95% C.I.**    92.4-99.4%

Of the 42 presumptive positives by the predicate device, 25 patients were further tested by PRNT and the EUROIMMUN Anti-West Nile Virus ELISA (IgM). The results are shown below:

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

Serum n = 25		PRNT Results		
		Positive	Borderline	Negative
<b>EUROIMMUN Anti- West Nile Virus ELISA (IgM)</b>	Positive	21	0	0
	Borderline	3	0	0
	Negative	1	0	0

**Sensitivity**                    84.0% (21/25)        **95% C.I.**    63.9-95.5%

**Clinical Study II:**

A study was performed at a clinical laboratory in the midwest, with 398 serum samples collected prospectively from patients suspected of infection with West Nile virus. The patients consisted of 193 men and 205 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 (IgM) in parallel with the predicate ELISA.

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

Serum n = 398		Predicate Assay		
		Positive	Borderline	Negative
<b>EUROIMMUN Anti- West Nile Virus ELISA (IgM)</b>	Positive	30	0	1
	Borderline	1	0	0
	Negative	2	0	364

**Positive Agreement** 90.9% (30/33) **95% C.I.** 75.7-98.1%  
**Negative Agreement** 99.7% (364/365) **95% C.I.** 98.5-100.0%

**Clinical Study III:**

A study was performed in cooperation with the public health agency/laboratory in Canada with 99 clinically collected serum samples, positive for West Nile virus IgM. The samples were tested by the predicate and the EUROIMMUN Anti-West Nile Virus ELISA (IgM). The results are shown below.

Clinical Study III: EUROIMMUN Anti-West Nile Virus ELISA (IgM) against predicate

Serum n = 99		Predicate Assay		
		Positive	Borderline	negative
<b>EUROIMMUN Anti- West Nile Virus ELISA (IgM)</b>	Positive	89	0	0
	Borderline	4	0	0
	Negative	6	0	0

**Positive agreement** 89.9% (89/99) **95% C.I.** 82.2-95.0%

4. Clinical cut-off:

Not Applicable

5. Expected Values:

Euroimmune assessed reactivity with 553 samples prospectively collected from patients in the US. The range of positivity of different populations from the US prospective studies with the EUROIMMUN Anti-West Nile Virus ELISA (IgM) test kit are presented below.

Expected Values from US Studies

<b>Age</b>	<b>n</b>	<b>Negative</b>	<b>Borderline</b>	<b>Positive</b>	<b>% Positive</b>	<b>95% C.I.</b>
<b>0-9</b>	16	16	0	0	0% (0/16)	0.0 – 20.6%
<b>10-19</b>	32	28	0	4	12.5% (4/32)	3.5 - 29.0%
<b>20-29</b>	66	65	0	1	1.5% (1/66)	0.0 - 8.2%
<b>30-39</b>	86	81	1	4	4.7% (4/86)	1.3 - 11.5%
<b>40-49</b>	89	79	0	10	11.2% (10/89)	5.5 - 19.7%
<b>50-59</b>	100	83	2	15	15.0% (15/100)	8.7 - 23.5%
<b>60+</b>	164	125	5	34	20.7% (34/164)	14.8 - 27.7%
<b>Total</b>	553	477	8	68	12.3% (68/553)	9.7 - 15.3%

**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.