

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
ASSAY ONLY TEMPLATE**

A. 510(k) Number:

k041817

B. Purpose for Submission:

New device clearance

C. Measurand:

West Nile Virus IgM Antibody

D. Type of Test:

Qualitative, ELISA

E. Applicant:

InBios International, Inc.

F. Proprietary and Established Names:

West Nile Detect IgM ELISA

G. Regulatory Information:

a) Regulation section:

West Nile Virus, serological reagents (21 CFR 866.3940).

b) Classification:

Class II

Product Code:

NOP

c) Panel:

83 Microbiology

H. Intended Use:

a) Intended use(s):

The West Nile *Detect*TM IgM Capture ELISA is for the qualitative detection of IgM antibodies to WNV recombinant antigens (WNRA) in serum for the presumptive clinical laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with meningoencephalitis. Positive results must be confirmed by Plaque Reduction Neutralization Test (PRNT), or by using the current CDC guidelines for diagnosis of this disease. Assay performance characteristics have not been established for testing cord blood, neonate, prenatal screening, general population screening without symptoms of

meningoencephalitis or automated instruments. This assay is not FDA cleared or approved for testing blood or plasma donors

- b) Indication(s) for use:
The West Nile *Detect*TM IgM Capture ELISA is for the laboratory diagnosis of West Nile Virus infection in patients with clinical symptoms consistent with meningoencephalitis.
- c) Special condition for use statement(s):
The device is for prescription use only
- d) Special instrument requirements:
NA

I. Device Description:

IgM Capture ELISA

J. Substantial Equivalence Information:

- a) Predicate device name(s):
Focus Technologies West Nile Virus IgM Capture ELISA
- b) Predicate K number(s):
K031952

Comparison with predicate:

Similarities		
Item	Device	Predicate
Same indications for use. Same target population. Same ELISA methodology	West Nile <i>Detect</i> TM IgM Capture ELISA (K041817)	Focus West Nile Virus IgM Capture ELISA (K031952)
	Test persons having symptoms of meningoencephalitis	Test persons having symptoms of meningoencephalitis
	IgM Capture ELISA	IgM Capture ELISA
Differences		
Item	Device	Predicate
Assay Procedure	West Nile <i>Detect</i> TM IgM Capture ELISA (K041817)	Focus West Nile Virus IgM Capture ELISA (K031952)
	Does specimen testing in duplicate	Does specimen testing in singlet

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:

The West Nile *Detect*TM IgM Capture ELISA consists of one enzymatically amplified "two-step" sandwich-type immunoassay. In this assay, controls and unknown serum samples are incubated in microtiter wells which have been coated with anti-human IgM antibodies, followed by incubation with West Nile Virus derived recombinant WNRA protein and a control preparation (NCA) separately. The serum samples may be directly mixed with sample dilution buffer for WN IgM added in the wells. After one hour incubation and washing, the wells are treated with a WNRA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate. An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbance of the WNRA and the control wells presumptively determines whether antibodies to WNV are present. A set of positive and negative controls is provided in order to monitor the integrity of the kit components.

M. Performance Characteristics (if/when applicable):Analytical performance:**a) Precision/Reproducibility:**

The reproducibility of the West Nile Detect IgM Capture ELISA was evaluated at three sites. One site was InBios International. Ten serum specimens using clinical specimens diluted into an analyte-negative matrix was used. The ten serum specimens (not including positive and negative controls) included specimens that were below the cutoff values (negative samples) and above the cutoff value (positive and weak positive or borderline samples). The serum dilutions selected also ensured that the analyte concentration in the specimens represented a clinically relevant range. The results are shown in the table below.

Reproducibility Results from three sites after deleting 2 outlying data points from Site #3

Sample ID	n	Mean	Intra-Assay		Between Day		Between Lab		Total	
			*S.D.	%CV	*S.D.	%CV	*S.D.	%CV	*S.D.	%CV
1	27	1.18	0.07	5.8%	0.14	11.4%	0.29	24.5%	0.33	27.6%
2	27	9.39	1.04	11.0%	3.10	33.1%	1.46	15.5%	3.58	38.2%
3	27	17.98	1.50	8.3%	4.00	22.2%	4.18	23.3%	5.98	33.2%
4	27	6.48	1.04	16.1%	1.98	30.5%	2.47	38.2%	3.33	51.4%
5	26*	21.07	2.54	12.0%	5.53	26.3%	7.56	35.9%	9.70	46.1%
6	27	7.92	0.74	9.4%	2.33	29.5%	3.46	43.7%	4.24	53.5%
7	26*	12.75	1.46	11.4%	2.21	17.3%	3.85	30.2%	4.67	36.6%
8	27	5.94	0.64	10.8%	1.62	27.3%	1.87	31.5%	2.56	43.1%

9									10.1	
	27	24.81	2.53	10.2%	8.86	35.7%	4.23	17.0%	4	40.9%
10	27	1.14	0.07	5.8%	0.04	3.2%	0.16	14.3%	0.18	15.8%

All values are calculated as WNRA/NCA ratios

SD = Standard Deviation; %CV = % Coefficient of Variation

26*: 1 statistically outlying (>5.5 x Standard Deviation of previous run) data point was removed.

a. **Linearity/assay reportable range:**

NA

b. **Traceability, Stability, Expected values (controls, calibrators, or method):**

NA

c. **Detection limit:**

NA

d. **Analytical specificity:**

Two hundred and seventy-one sera that tested positive for other potentially cross-reactive pathogens were tested with the West Nile Detect IgM Capture ELISA test to determine the potential for cross-reactivity. The table below summarizes the results of this study.

Disease	Number of Samples	West Nile Detect IgM ELISA		Total of Positive and Equivocal
		Equivocal	Positive	
Eastern Equine encephalitis	17	0	0	0/17
Japanese encephalitis	2	0	0	0/2
Saint Louis encephalitis	32	1	16	17/32
La Crosse Virus	6	0	0	0/6
Dengue virus	7	0	2	2/7
Epstein-Barr virus	15	0	0	0/15
Hepatitis A virus	10	0	0	0/10
Hepatitis B virus	49	0	0	0/42
Hepatitis C virus	30	0	0	0/20
Herpes simplex virus	32	0	0	0/32
California Encephalitis (CE)	1	0	0	0/1
HIV	20	0	0	0/20
Syphilis	5	0	0	0/5
Cytomegalovirus	12	0	0	0/12
Varicella zoster virus	10	0	0	0/10
Coxsackievirus B 1-6	1	0	0	0/1
Echovirus 16	1	0	0	0/1
Measles	1	0	0	0/1
Mumps	1	0	0	0/1
Polio Blend	1	0	0	0/1
Legionaries' disease	3	0	0	0/3
Rheumatoid factor	5	0	0	0/5

Anti-nuclear antibody	10	0	0	0/10
Total	271	1	18	19/271

Caution: IgM assay cross-reactivity has been noted with some West Nile IgM assays testing specimens containing antibody to enteroviruses. Reactive results reported from children must contain a caution statement regarding possible cross-reactivity with enteroviruses.

e. Assay cut-off:

The cut-off was selected using sera from an endemic population in the United States. The 282 samples consisted of 163 positive samples and 119 negative samples characterized by the CDC IgM Antibody Capture ELISA. The cut-off was determined by two-graph receiver operating characteristic analysis (TG-ROC).

f. Comparison studies:

a. Method comparison with predicate device:

The West Nile *Detect*TM IgM Capture ELISA was compared to two reference assays: The plaque-reduction neutralization test (PRNT) and the CDC IgM Antibody Capture ELISA

b. Matrix comparison:

NA

c. Clinical sensitivity:

NA

d. Clinical specificity:

NA

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

PERFORMANCE CHARACTERISTICS

a. Clinical Studies:

Table 1 – Study Site 1

A clinical laboratory located in the mid-western U.S. tested 50 retrospective samples with clinically and laboratory confirmed cases of WNV (n=50) or undetermined flavivirus (positive for both WNV and SLE; n=2). The samples were suspected to have come from

patients that had exhibited signs or symptoms of WN but specific clinical information could not be confirmed. In addition, 125 retrospective sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Two were confirmed with undetermined flavivirus or WNV by PRNT.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	50	2	0	52
Negative	1	121	1	123
Total	51	123	1	175

WN Virus Positive:

Serological Sensitivity = $50/52 = 96.2\%$ 95% Confidence Interval: 87.0 – 98.9%

WN Virus Negative:

Serological Specificity = $121/123 = 98.4\%$ 95% Confidence Interval: 94.3 - 99.6%

Table 2 – Study Site 2

A State Department of Health laboratory located in Midwestern U.S. tested 88 retrospective samples clinically and laboratory confirmed cases of WNV and/or SLE and confirmed by PRNT. Seven patient samples were suspected of having either viral encephalitis or viral meningitis. The remaining patient samples had signs or symptoms of WN fever and headache. In addition, 130 retrospective, sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Fourteen (14) were confirmed with SLE and/or WNV by PRNT.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	99	2	1	102
Negative	1	115	0	116
Total	100	117	1	218

WN Virus Positive:

Clinical Sensitivity = $99/102 = 97.1\%$ 95% Confidence Interval: 91.7 – 99.0%

WN Virus Negative:

Clinical Specificity = $115/116 = 99.1\%$ 95% Confidence Interval: 95.3 - 99.9%

Table 3 – Study Site 3

A state department of health laboratory located in Southeastern U.S. tested 150 retrospective samples clinically and laboratory confirmed cases of WNV by PRNT. In addition, 150 retrospective, sequential endemic samples were tested. The sera were sequentially submitted to the laboratory, archived, and masked. Twenty-three (23) were confirmed with SLE and/or WNV by CDC ELISA.

Clinical Category	Positive	Negative	Equivocal	Total
PRNT Positive	172	1	0	173
Negative	0	127	0	127
Total	172	128	0	300

WN Virus Positive:

Serological Sensitivity = $172/173 = 99.4\%$ 95% Confidence Interval: 96.8 – 99.9%

WN Virus Negative:

Serological Specificity = $127/127 = 100.0\%$ 95% Confidence Interval: 97.1 - 100%

Study Site 4

A state department of health laboratory located in Northeastern U.S. tested 210 retrospective, sequential endemic samples with the West Nile Detect IgM Capture ELISA and with the CDC MAC ELISA. The sera were sequentially submitted to the laboratory, archived, and masked. None of the samples gave a positive result with both tests.

Clinical Category	Positive	Negative	Equivocal	Total
CDC MAC ELISA				
Positive	0	0	0	0
Negative	0	210	0	210
Total	0	210	0	210

Negative Presumptive Agreement

$210/210 = 100.0\%$ 95% Confidence Interval: 98.2 – 100.0%

b. Clinical cut-off:

NA

c. Expected values/Reference range:

Expected Values

West Nile virus infection is generally recognized by the presence of IgM antibodies within one week from the beginning of symptoms. Detectable levels of IgM may be low in early infection. Two hundred samples prospectively collected from Florida, Texas and Pennsylvania during March 2004. The distribution of females was 50% (100/200) and males were 50% (100/200). The data in Table 1 illustrates the prevalence of IgM antibodies in different age groups when using the West Nile Detect IgM Capture ELISA Test.

Of the 200 normal sera, one was positive and one was equivocal. The latter specimen was repeated in duplicate and remained equivocal on the West Nile Detect IgM Capture ELISA Test. The positive and equivocal sera were from Pennsylvania. Of the 200 sera, 66 were from Pennsylvania, resulting in a 3.0% prevalence (2/66) in Pennsylvania.

Table 1

Age	Total	Equivocal	Positive	Prevalence
10-20	12	0	0	0.0%
21-30	68	1	0	1.5%
31-40	63	0	0	0.0%
41-50	47	0	1	2.1%
51-60	10	0	0	0.0%
Total	200	1	1	1.0%

N. Proposed Labeling:

The labeling is sufficient and satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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