510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE

A. 510(k) Number:
k031952

B. Analyte:
West Nile Virus IgM Antibody

C. Type of Test:
Qualitative, ELISA

D. Applicant:
Focus Technologies, Inc

E. Proprietary and Established Names:
West Nile Virus IgM Capture ELISA

F. Regulatory Information:

1. Regulation section:
   West Nile Virus, serological reagents (21 CFR 866.3940).
2. Classification:
   Class II
3. Product Code:
   NOP
4. Panel:
   Microbiology (83)

G. Intended Use:

1. Intended use(s):
   The Focus Technologies West Nile Virus IgM Capture ELISA is intended for qualitatively detecting IgM antibodies to West Nile virus in human serum. In conjunction with the Focus Technologies West Nile Virus ELISA IgG, the test is indicated for testing persons having symptoms of meningioencephalitis, as an aid in the presumptive clinical 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 or approved for testing blood or plasma donors. Assay performance characteristics have not been established for automated instruments
2. Indication(s) for use:
   The West Nile Virus IgM Capture ELISA is for the laboratory diagnosis of West Nile Virus infection in patients with clinical symptoms consistent with meningitis/encephalitis.
3. Special condition for use statement(s):
   Not Applicable
4. Special instrument Requirements:
   Not Applicable

H. Device Description:
IgM Capture ELISA

I. Substantial Equivalence Information:

1. Predicate device name(s):
   PanBio West Nile Virus IgM Capture ELISA
2. Predicate K number(s):
   K031703
3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Focus West Nile Virus IgM Capture ELISA	PanBio West Nile Virus IgM ELISA
Same indications for use. Same target population.	Test persons having symptoms of meningioencephalitis	Test persons having symptoms of meningioencephalitis
Same ELISA methodology	IgM Capture ELISA	IgM Capture ELISA
Differences		
Item	**Device**	**Predicate**
	Focus West Nile Virus IgM Capture ELISA	PanBio West Nile Virus IgM ELISA
Different WNV antigens used in the assay	Recombinant antigen	Inactivated native virus

J. Standard/Guidance Document Referenced (if applicable):
Not Applicable

K. Test Principle:

In the Focus Technologies West Nile Virus IgM Capture ELISA, the polystyrene microwells are coated with anti-human antibody specific for IgM (μ-chain). Diluted serum samples and controls are incubated in the wells, and IgM present in the sample binds to the anti-human antibody (IgM specific) in the wells. Non-specific reactants are removed by washing. WNV antigen is then added to the wells and incubated; and, if anti-WNV IgM is present in the sample, the WNV antigen binds to the anti-WNV in the well. Unbound WNV antigen is then removed by washing the well. Mouse anti-flavivirus conjugated with horseradish peroxidase (HRPO) is then added to the wells and incubated; and, if WNV antigen has been retained in the well by the anti-flavivirus in the sample, the mouse anti-flavivirus: HRPO binds to the WNV antigen in the wells. Excess conjugate is removed by washing. Enzyme substrate and chromogen are added, and the color is allowed to develop. After adding the Stop Reagent, the resultant color change is quantified by a spectrophotometric reading of optical density (OD) that is directly proportional to the amount of antigen-specific IgM present in the sample. Sample optical density readings are compared with reference cut-off OD readings to determine results.

L. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. Precision/Reproducibility:

Reproducibility studies included Inter-lot Reproducibility, Inter/Intra-assay Reproducibility, and Inter-laboratory Reproducibility. In each study, two sets of samples were masked duplicates. Focus assessed the device's Inter-lot Reproducibility by testing five samples on three separate days with three separate lots. For one lot, the samples were run in triplicate, and run in duplicate with the other two lots. Each of the three lots had a different lot of Antigen and Capture Wells. Focus assessed the device's Inter/Intra-assay Reproducibility by testing seven samples in triplicate, once a day, for three days, for a total of 63 data points. A state department of health laboratory located in the northeastern U.S. (Study Site 1), a clinical laboratory located in the mid-western U.S. (Study Site 2), and Focus assessed the device's Inter-laboratory Reproducibility. Each of the three laboratories tested seven samples in triplicate on three different days.

Reproducibility

Sample	Inter- & Intra-assay			Inter-lot		Inter-Lab	
	Index Mean	Intra-assay %CV	Inter-assay %CV	Index Mean	Index %CV	Index Mean	Index %CV
M2*	0.21	2.9	10.3	0.22	1.2	0.23	9.7
M6*	0.23	3.4	20.0	0.23	0.4	0.24	13.2
M5	0.69	1.6	5.7	0.70	0.7	0.71	6.4
M1*	1.43	1.5	2.9	1.41	2.6	1.45	4.0
M7*	1.53	1.8	4.0	1.54	2.1	1.49	12.8
M3	2.37	2.7	1.7	2.33	3.6	2.23	2.5
M4	2.99	1.9	0.3	2.98	1.9	2.78	2.3

* There were two sets of masked pairs (same sample, different labeled identity): M2 & M6 were one masked pair, and M1 & M7 were the second masked pair.

b. Linearity/assay reportable range:
Not Applicable

c. Traceability (controls, calibrators, or method):
Not Applicable

d. Detection limit:
Not Applicable

e. Analytical specificity:

Focus and a state department of health laboratory located in the northeastern U.S. (DOH) (Study Site 1) assessed the device's cross-reactivity with sera that were sero-positive to other potentially cross-reactive pathogens (n = 75). The DOH tested the SLE positives, and Focus tested the other sera. The sera were retrospective and masked. The following table summarizes the cross-reactivity data.

Cross-reactivity

Population	Site	Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	14	1	5	15	40.0% (6/15) 95%CI=16.3 to 67.7%
St. Louis encephalitis virus	1	6	0	7	13	53.8% (7/13) 95%CI 25.1-80.8%
Eastern Equine Encephalitis virus	4	2	0	0	2	0.0% (0/2) 95%CI 0.0-84.2%
Herpes simplex virus	4	18	1	1	20	10.0% (2/20) 95%CI: 1.2-31.7%
Epstein-Barr virus	4	19	0	0	19	0.0% (0/19) 95%CI 0.0-17.6%
Cytomegalovirus	4	13	0	1	14	7.1% (1/14) 95%CI 0.2-33.9%
Borrelia burgdorferi	4	0	0	3	20	15.0% (3/20) 95%CI3.2-37.9%
Rheumatoid factor	4	0	1	4	20	25.0% (5/20) 95%CI 3.7-49.1%
Anti-nuclear antibodies	4	0	0	1	20	5.0% (1/20) 95%CI 0.1-24.9%

Because of the high degree (25%) of cross-reactivity with specimens containing Rheumatoid factor, the following warning has been placed in the package insert.

Caution: IgM assay cross-reactivity has been noted with specimens containing rheumatoid factor (RF). All reactive results must be reported with a caution statement regarding possible cross-reactivity with RF.

f. Assay cut-off:

In designing the assay, the assay Cut-off was established to optimize both sensitivity and specificity by using 315 sera submitted for West Nile testing consisting of 4 different serum panels: 1) 98 confirmed acute West Nile positive samples (PRNT positives); 2) 102 presumed acute West Nile positive samples (US Public Health IgM ELISA and/or in-house WNV antigen IgM ELISA positive); 3) 108 presumed negative samples (US Public Health IgM ELISA and/or in-house WNV native antigen IgM ELISA negative); and 4) 7 ELISA discrepant s(US Public Health IgM ELISA negative and in-house WNV native antigen IgM ELISA positive). The Focus West Nile IgM was: positive with 99.0% (96/97) of the confirmed acute West Nile positive samples (excluding one equivocal); positive with 99.0% (101/102) of the presumed acute West Nile positive samples: negative with 100% (108/108) of the presumed negative samples; and negative with 71.4% (5/7) of the ELISA discrepant samples.

2. Comparison studies:
    a. *Method comparison with predicate device:*
    The Focus IgM Capture ELISA was compared with two reference assays: The plaque-reduction neutralization test (PRNT) and the CDC MAC ELISA.
    b. *Matrix comparison:*
    Not Applicable
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):*

Study Site 1: Focus Reactivity with Encephalitis/Meningitis Patients (n = 300)

A state department of health laboratory located in the northeastern U.S. assessed the device's reactivity from encephalitis/meningitis patients (n = 300). Patients were suspected of having either viral encephalitis or viral meningitis. Viral encephalitis criteria included: 1) fever; 2) altered mental status and/or other evidence of cortical involvement; and 3) CSF pleocytosis with predominant lymphocytes and/or elevated protein and a negative gram stain and culture. Viral meningitis criteria included: 1) fever; 2) headache, stiff neck and/or other meningeal signs; and 3) CSF pleocytosis with predominant lymphocytes and/or elevated protein and a negative gram stain and culture). The sera were sequentially submitted to the laboratory, archived, and masked. The reference methods were the CDC IgM ELISA, and a plaque reduction neutralization test (PRNT) for West Nile virus.

Of 300 encephalitis/meningitis patients, 44 were classified as confirmed positive West Nile encephalitis patients (encephalitis/meningitis symptoms, CDC IgM ELISA positive and WNV PRNT positive) and 256 had presumptive assay results (CDC WNV IgM ELISA). The Focus IgM assay was positive with 90.9% (40/44) of the confirmed positive WNV encephalitis patients (including 2 Focus equivocals calculated as negatives). Of the 256 patients with presumptive assay results, 254 were classified as presumed negative patients (CDC WNV IgM ELISA negative),), and 2 were classified as presumed positive West Nile encephalitis patients (CDC WNV IgM ELISA positive). The Focus IgM assay was positive with 100% (2/2) of the presumed positive WNV encephalitis patients. The Focus IgM assay was negative with 98.8% (251/254) of the presumed negative patients (including 2 Focus equivocals calculated as positives).

Study Site 1: Focus Reactivity with Encephalitis/Meningitis Patients (n=300)

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	**Eqv**	**Pos**	**Total**	**%**
Clinical sensitivity (encephalitis or meningitis symptoms, CDC IgM ELISA positive and WNV PRNT positive)	2	2	40	44	90.9% (40/44) 95%CI 78.3-97.5%
Agreement with the presumptive CDC IgM ELISA	251	2	3	256	Positive 100% (2/2) 95%CI 15.8-100% Negative 98.8% (251/254) 95%CI 96.6-99.8%

Study Site 2: Focus Reactivity with WNV PRNT Positives (n = 75)

A clinical laboratory located in the mid-western U.S. assessed the device's reactivity with 75 retrospective samples with no clinical information that were pre-screened positive (by Focus) with a West Nile virus native antigen ELISA, and confirmed West Nile positive by plaque reduction neutralization test (PRNT). The sera were sequentially submitted to the laboratory, archived, and masked. The Focus IgM ELISA was positive with 100% (75/75) of the WNV PRNT positive samples**.**

Study Site 2: Focus Reactivity with WNV PRNT Positives (n = 75)

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	**Eqv**	**Pos**	**Total**	**%**
Serological sensitivity (WNV PRNT positive)	0	0	75	75	100% (75/75) 95%CI 95.2-100%

Study Site 3: Focus Reactivity with West Nile IFA Negatives (n=103)

A clinical laboratory located in the southwestern U.S. assessed reactivity with 103 retrospective samples that were West Nile IFA negative. The Focus IgM ELISA was negative with 96.1% (99/103) of WNV IgM IFA negative samples (including one equivocal calculated as positive).

Study Site 3: Focus Reactivity with West Nile IFA Negatives (n=103)

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	Eqv	Pos	Total	%
Negative agreement with presumptive WNV IFA	99	1	3	103	96.1% (99/103) 95%CI 90.3-98.9%

Study Site 4: Focus Reactivity with Suspected Encephalitis/Meningitis Patients (n= 50)

Focus assessed the device's reactivity with 50 samples from patients suspected of encephalitis/meningitis. A U.S. federal government laboratory provided the archived and masked sera. One sample was confirmed positive by WNV PRNT, and the other 49 were presumptively negative (CDC ELISA) for arboviruses present in North America (La Crosse virus, Eastern Equine encephalitis virus, Saint Louis encephalitis virus and WNV). The Focus IgM ELISA was negative with 98.0% (48/49) of the WNV presumptive negative samples, and positive with the one WNV PRNT confirmed sample.

Study Site 4: Reactivity with Suspected Encephalitis/Meningitis Patients (n= 50)

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	Eqv	Pos	Total	%
Serological sensitivity (CDC IgM ELISA positive and WNV PRNT positive)	0	0	1	1	100% (1/1) 95%CI NA
Negative agreement with presumptive CDC IgM ELISA	48	0	1	49	98.0% (48/49) 95%CI 89.1-99.9%

Study Site 4: Focus Reactivity with Non-Flavivirus Test Samples (n = 476)

Focus assessed the device's reactivity with 476 samples prospectively collected from North America during August 2003. The samples had been submitted to a clinical laboratory located in Southern California for testing for infectious diseases. Positive samples were tested with a CDC WNV IgM ELISA. The Focus West Nile IgM Capture ELISA was negative with 99.4% (468/471) of the CDC ELISA IgM negative samples (including 3 Focus equivocals included as positive), and positive with 100% (1/1) of the CDC ELISA IgM positive samples.. Four CDC ELISA IgM indeterminant samples were excluded from the calculations.

Study Site 4: Focus Reactivity with Non-Flavivirus Test Samples (n = 476)*

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	Eqv	Pos	Total	%
Positive agreement with presumptive CDC IgM ELISA	0	0	1	1	100% (1/1) 95%CI NA
Negative agreement with presumptive CDC IgM ELISA	468	3	0	471	99.4% (468/471) 95%CI 98.1-99.9%

4. Clinical cut-off:
   Not Applicable
5. Expected values/Reference range:

The prevalence of West Nile antibodies varies depending on age, geographic location, testing method used, and other factors. A community based serosurvey for West Nile infection conducted in New York in 2000 found that 0.2% (5/2433) of persons tested overall had antibodies indicating recent West Nile infection, and that 1.1% (2/176) of persons reporting a recent headache and fever had antibodies indicating a recent West Nile infection. Two serosurveys conducted in New York City (NYC) in 1999 and 2000 showed that approximately 1 in 150 infections (<1%) resulted in meningitis or encephalitis. The NYC results are consistent with a 1996 Romanian serosurvey indicating that 1:140 to 1:320 infections resulted in meningitis or encephalitis.

Prevalence in Samples Submitted for Non-Flavivirus Testing (n=476)

Focus assessed reactivity with 476 samples prospectively collected from North America during August 2003. The samples had been submitted to a clinical laboratory located in Southern California for non-flavivirus tests (e.g., tests for other infectious diseases). The samples consisted of 64.1% females, 34.5% males, and 1.5% from persons of unspecified gender.

IgM Prevalence with Samples Submitted for Non-Flavivirus Testing (n=476)

Focus WNV IgM ELISA					
Age	Neg	Eqv	Pos	% Positive	95%CI
0 to 9	24	0	0	0.0% (0/24)	0.0-14.2%
10 to 19	28	0	1	3.5% (1/29)	0.1-17.8%
20 to 29	70	0	0	0.0% (0/70)	0.0-5.1%
30 to 39	82	0	0	0.0% (0/82)	0.0-4.4%
40 to 49	77	0	1	1.3% (1/78)	0.0-6.9%
50 to 59	48	1	2	3.9% (2/51)	0.5-13.5%
60 to 69	38	0	1	2.6% (1/39)	0.1-13.5%
70 to 79	34	0	0	0.0% (0/34)	0.0-10.3%
80+	17	1	0	0.0% (0/18)	0.0-18.5%
Unknown	50	1	0	0.0% (0/51)	0.0-7.0%
Overall	468	3	5	1.1% (5/476)	0.3-2.4%

M. Conclusion:

The data demonstrated that there was very good agreement between the reference assays and the Focus West Nile Virus IgM Capture ELISA. It is believed that the above information demonstrates that the Focus West Nile Virus IgM Capture ELISA is substantially equivalent to the PanBio West Nile Virus IgM ELISA. When the Focus West Nile Virus IgM Capture ELISA is used according to its directions for use, it should be safe and effective for the indications for use claimed.