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

A. 510(k) Number:
k040854

B. Purpose for Submission:
A background subtraction step was added to the assay procedure and the rheumatoid factor warning has been modified. The background subtraction procedure has been added to the ELISA procedure so that all positives results must be run with the background subtraction to eliminate false positives due to cross-reacting antibodies.

C. Analyte:
West Nile Virus IgM Antibody

D. Type of Test:
Qualitative, ELISA

E. Applicant:
Focus Technologies, Inc

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

G. 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)

H. 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 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 or approved for testing blood or plasma donors. Assay performance characteristics have not been established for automated instruments

2. Indication(s) for use:
The Focus Technologies 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

I. Device Description:

IgM Capture ELISA

J. Substantial Equivalence Information:

1. Predicate device name(s):
Focus Technologies West Nile Virus IgM Capture ELISA

2. Predicate K number(s):
K031952

3. Comparison with predicate:

Similarities		
Item	**Device**	**Predicate**
	Focus West Nile Virus IgM Capture ELISA (K040854)	Focus West Nile Virus IgM ELISA (K031952)
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 (K040854)	Focus Nile Virus IgM ELISA (K031952)
	Uses background method	Does not use Background

Assay Procedure	on positive results in Assay Procedure	method in the Assay Procedure

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 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.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. ***Precision/Reproducibility:***

Focus, a clinical laboratory located in the mid-west United States and a university laboratory located in northern California assessed the reproducibility of the assay with and without the background subtract procedure. Each laboratory tested seven samples in triplicate in three runs per day for three days. Of the seven samples, three samples were negative (BS1, BS2 and BS6), two samples were positive in the assay and with background subtract (BS22 and BS3), and two samples were positive in the assay but negative in background subtract (BS21 and BS23, these samples were masked replicates). The results of the studies are summarized in the tables below:

Focus Reproducibility without Background Subtract

ID	Mean Index	Inter-Lab %CV	Inter-assay %CV	Intra-assay %CV	Total %CV
BS1	0.06	36.9	42.5	15.4	47.8
BS6	0.07	22.5	31.2	13.2	31.2
BS2	0.09	15.1	27.6	14.7	30.5
BS22	1.49	1.5	5.2	3.0	5.7
BS3	2.49	3.6	6.2	3.7	6.7
BS21*	2.72	24.4	23.3	4.3	29.7
BS23*	2.75	25.3	24.0	2.6	30.5

* These samples were masked replicates

Focus Reproducibility with Background Subtract

ID	Mean Index	Inter-Lab %CV	Inter-assay %CV	Intra-assay %CV	Total %CV
BS1	NA	NA	NA	NA	NA
BS6	NA	NA	NA	NA	NA
BS2	NA	NA	NA	NA	NA
BS22	1.46	2.1	7.6	3.4	7.9
BS3	2.47	1.2	8.6	3.6	8.9
BS21*	-0.08	-92.0	-198.3	-351.7	-224.7
BS23*	-0.06	-41.8	-194.2	-127.6	-238.3

* These samples were masked replicates

b. Linearity/assay reportable range:
Not Applicable

c. Traceability, Stability, Expected values (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) 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.

Focus Cross-reactivity without Background Subtract

Specimens characterized by Reference Assays	Site	Focus WNV IgM ELISA Results				
		Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	6	1	8	15	40.0% (6/15) 95%CI 16.3-67.7%
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%

Focus Cross-reactivity with Background Subtract

Specimens characterized by Reference Assays	Site	Focus WNV IgM ELISA Results				
		Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	9	3	3	15	40.0% (6/15) 95%CI 16.3-67.7%
St. Louis encephalitis virus*	NA	NA	NA	NA	NA	Not tested.
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	1	20	5.0% (1/20) 95%CI 0.1-24.9%
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%
Polio virus	4	10	0	0	10	0.0% (0/10) 95%CI 0.0-30.8%

Herpes simplex virus	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Epstein-Barr virus	4	19	0	0	19	0.0% (0/19) 95%CI 0.0-17.6%
Cytomegalovirus	4	13	0	1	14	0.0% (0/14) 95%CI 0.0-23.2%
Borrelia burgdorferi	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Rheumatoid factor	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Anti-nuclear antibodies	4	20	0	0	20	0.0% (0/20) 95%CI: 0.0-16.8%
Polio virus	4	10	0	0	10	0.0% (0/10) 95%CI 0.0-30.8%

* Positive SLE samples were not tested with the background subtract procedure.

f. Assay cut-off:
Not Applicable

2. Comparison studies:

a. Method comparison with predicate device:
The Focus IgM Capture ELISAs 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 ELISAs, and a plaque reduction neutralization test (PRNT) for West Nile virus.

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

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

Without the Background Subtraction

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	249	1	0	250	Positive 100% (2/2) 95%CI 15.8-100% Negative 99.6% (249/250) 95%CI 97.8-100%

With the Background Subtraction

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	1	41	44	93.2% (41/44) 95%CI 78.3-97.5%
Agreement with the presumptive CDC IgM ELISA	250	0	0	250	Positive 100% (2/2) 95%CI 15.8-100% Negative 100% (250/250) 95%CI 98.6-100%

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.

Without Background Subtract

The clinical laboratory located in the mid-western U.S. determined that the Focus IgM ELISA was positive with 100% (75/75) of the WNV PRNT positive samples.

With Background Subtract

Focus determined that the Focus IgM ELISA was positive with 100% (70/70) of the WNV PRNT positive samples. Five samples were QNS for the background subtract procedure.

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 2: Focus Reactivity with WNV PRNT Positives (n = 70)*

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results				
	Neg	Eqv	Pos	Total	%
Serological sensitivity (WNV PRNT positive)	0	0	70	70	100% (70/70) 95%CI 94.9-100%

* Five of the 75 samples were QNS.

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.

Without Background Subtract

The Focus IgM ELISA was negative with 96.1% (99/103) of WNV IgM IFA negative samples (including one equivocal calculated as positive).

With Background Subtract

The Focus IgM ELISA was negative with 98.1% (101/103)) 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 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	101	1	1	103	98.1% (101/103) 95%CI 93.2-99.8%

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 (LAC), Eastern Equine Encephalitis (EEE), St. Louis Encephalitis (SLE) and WNV).

Without Background Subtract

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%

With Background Subtract

The Focus IgM ELISA was negative with 100% (49/49) 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	49	0	0	49	100% (49/49) 95%CI 92.7-100%

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

Without Background Subtract

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 33.3% (1/3) 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	2	1	3	33.3% (1/3) 95%CI 0.8-90.6%

With Background Subtract

The Focus West Nile IgM Capture ELISA was negative with 100% (469/469) of the CDC ELISA IgM negative samples, and positive with 66.7% (2/3) 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	1	0	2	3	66.7% (2/3) 95%CI 9.4-99.2%

Negative agreement with presumptive CDC IgM ELISA	468	1	0	469	99.8% (468/469) 95% CI 98.8–100%

* Excludes four samples that were indeterminant with the CDC IgM ELISA.

Negative agreement with presumptive CDC IgM ELISA	469	0	0	469	100% (469/469) 95% CI 99.2-100%

* Excludes four samples that were indeterminant with the CDC IgM ELISA.

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

Prevalence in Samples Submitted for Non-Flavivirus Testing (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 and/or the CDC WNV IgG ELISA.

IgM Prevalence with Samples Submitted for Non-Flavivirus Testing without Background Subtract (n=476)

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%

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

Age	Neg	Eqv	Pos	% Positive	95%CI
0 to 9	24	0	0	0.0% (0/24)	0.0-14.2%
10 to 19	29	0	0	0.0% (0/29)	0.0-11.9%
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	78	0	0	0.0% (0/78)	0.0-4.6%
50 to 59	51	0	0	0.0% (0/51)	0.0-7.7%
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	0	1	5.6% (1/18)	0.1-27.3%
Unknown	51	0	0	0.0% (0/51)	0.0-7.0%
Overall	474	0	2	0.4% (2/476)	0.1-1.5%

N. Conclusion:

The above information demonstrates that the new Focus West Nile Virus IgM Capture ELISA (K040854) is substantially equivalent to the previous cleared Focus West Nile Virus IgM ELISA (K031952). The data demonstrated that there was good agreement between the two Focus West Nile Virus IgM Capture ELISA. The new Focus assay (K040854) will decrease the number of false positive results due to cross-reactive antibodies. When the new 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.

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