

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

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

k031953

**B. Analyte:**

West Nile Virus IgG Antibody

**C. Type of Test:**

Qualitative, ELISA

**D. Applicant:**

Focus Technologies, Inc

**E. Proprietary and Established Names:**

West Nile Virus ELISA IgG

**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 ELISA IgG is intended for qualitatively detecting IgG antibodies to West Nile virus in human serum. In conjunction with the Focus Technologies West Nile Virus IgM Capture ELISA, 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.
2. Indication(s) for use:  
The West Nile Virus ELISA IgG 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:**

Indirect IgG 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:

<b>Similarities</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Same indications for use.  Same target population.	Focus West Nile Virus ELISA IgG  The test is indicated for testing persons having symptoms of meningoencephalitis	PanBio West Nile Virus IgM Capture ELISA  For the laboratory diagnosis of West Nile virus infection in patients with clinical symptoms consistent with meningitis/encephalitis.
<b>Differences</b>		
<b>Item</b>	<b>Device</b>	<b>Predicate</b>
Different antigens used in the assay  Different ELISA methodology	Focus West Nile Virus IgM Capture ELISA  Recombinant antigen  Indirect ELISA	PanBio West Nile Virus IgM ELISA  Inactivated native virus  IgM Capture ELISA

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

Not Applicable

**K. Test Principle:**

In the Focus Technologies West Nile Virus ELISA IgG assay, the polystyrene microwells are coated with recombinant West Nile virus antigen. Diluted serum samples and controls are incubated in the wells to allow specific antibody present in the samples to react with the antigen. Nonspecific reactants are removed by washing, and peroxidase-conjugated anti-human IgG is added and reacts with specific IgG. 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). 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 (Study Site 4) 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 (Study Site 4) 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), and a clinical laboratory located in the mid-western U.S. (Study Site 2), Focus (Study Site 4), assessed the device's Inter-laboratory Reproducibility. Each of the three laboratories tested seven samples in triplicate on three different days.

Sample	Inter- & Intra-assay			Inter-lot		Inter-Lab	
	Index Mean	Intra-assay %CV	Inter-assay %CV	Index Mean	Index %CV	Index Mean	Index %CV
G6*	0.23	12.2	18.2	0.30	13.1	0.32	11.6
G2*	0.29	21.2	17.3	0.34	7.5	0.35	22.5
G5	0.65	7.9	21.3	0.73	7.2	0.69	19.0
G7*	1.14	3.5	18.2	1.30	5.7	1.21	14.1
G1*	1.22	3.2	17.1	1.36	7.0	1.25	16.4
G4	2.44	1.0	16.2	2.79	4.1	2.47	12.8
G3	2.98	3.9	17.3	3.37	4.1	3.10	12.6

\* There were two sets of masked pairs (same sample, different labeled identity): G2 & G6 were one masked pair, and G1 & G7 were the second masked

###### 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 seropositive to other potentially cross-reactive pathogens (n = 75). The DOH tested the SLE positives, and Focus tested the other sera. The sera were archived and masked. The table below summarizes the data.

## Cross-reactivity

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results					
	Site	Neg	Eqv	Pos	Total	% Positive
Dengue virus (secondary infections)	4	1	0	19	20	95.0% (19/20) 95% CI 75.1-99.9%
Japanese encephalitis virus	4	14	3	3	20	30.0% (6/20) 95% CI 11.9-54.3%
St. Louis encephalitis virus	1	8	1	11	21	57.1% (12/21) 95% CI 34.0-78.2%
Yellow fever virus	4	11	4	5	20	45.0% (9/20) 95% CI 23.1-68.5%
Alphavirus (Sindbis & Eastern equine viruses)	4	15	0	2	17	11.8% (2/17) 95% CI 0.1-36.4%
Bunyavirus (Jamestown Canyon & La Crosse)	1	12	2	1	15	20.0% (3/15) 95% CI 4.3-48.1%
Herpes simplex virus type 1	4	55	0	5	60	8.3% (5/60) 95% CI 2.8-18.4%
Epstein-Barr virus	4	11	0	1	12	8.3% (1/12) 95% CI 0.2-38.5%
Cytomegalovirus	4	16	0	4	20	20.0% (4/20) 95% CI 5.7-43.7%
Echovirus/Poliovirus	4	18	1	1	20	10.0% (2/20) 95% CI 1.2-31.7%
<i>Borrelia burgdorferi</i> (Lyme disease)	4	17	1	2	20	15.0% (3/20) 95% CI 3.2-37.9%

Because of the high degree of cross-reactivity with specimens containing antibodies to CMV and bunyaviruses, the following warning has been placed in the package insert.

**Caution: IgG assay cross-reactivity has been noted with some specimens containing antibody to cytomegalovirus (CMV) and bunyaviruses, e.g., LaCrosse virus. Reactive results must be reported with a caution statement regarding possible cross-reactivity with CMV and bunyaviruses, e.g., La Crosse virus.**

*f. Assay cut-off:*

**Cut-off Development.** In designing the assay, the assay Cut-off was established to slightly favor specificity over sensitivity by using 217 sera consisting of 3 different serum panels: 1) 136 sera submitted for West Nile testing and positive with an in-house WNV IgG ELISA native antigen West Nile ELISA IgG); 2) 61 sera submitted for West Nile testing and negative with an in-house WNV IgG ELISA; and 3) 20 blood donors. The Focus West Nile IgG was positive with 90.4% (122/135) of the in-house WNV IgG ELISA positive samples, negative with 100% (61/61) of the in-house WNV IgG ELISA negative samples, and negative with 100% (20/20) of the blood donor samples.

2. Comparison studies:

*a. Method comparison with predicate device:*

The Focus West Nile Virus ELISA IgG was compared with two reference assays: The plaque-reduction neutralization test (PRNT) and the CDC IgG 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 CDC IgG ELISA, and plaque reduction neutralization test (PRNT) for West Nile virus. Of 300 encephalitis/meningitis patients, 205 were classified as presumed negative patients (CDC IgG ELISA negative), 37 classified as confirmed positive West Nile encephalitis patients (CDC IgG ELISA positive, WNV PRNT

positive), 4 presumed positive flavivirus encephalitis patients (CDC IgM positive, PRNT negative), one confirmed dengue positive (CDC IgG ELISA positive, dengue PRNT positive), and 53 unclassified because the CDC IgG ELISA results were indeterminate or equivocal. The Focus IgG ELISA was positive with 97.3% (36/37) of the confirmed positive WNV encephalitis patients (including 1 Focus equivocal calculated as negative). The Focus IgG ELISA was positive with 100% (4/4) of the presumed positive WNV encephalitis patients, and positive with the one dengue positive patient. The Focus IgG ELISA was negative with 99.0% (203/205) of the presumed negative patients (including 1 Focus equivocal counted positive). The 53 unclassified patients were excluded from the calculations.

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

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results				
	Neg	Eqv	Pos	Total	%
Clinical sensitivity (encephalitis or meningitis symptoms, CDC IgG ELISA positive and WNV PRNT positive)	0	1	36	37	97.3% (36/37) 95%CI 85.8-99.9%
Agreement with the presumptive CDC IgG ELISA	203	1	6	210	<u>Positive</u> † 100% (5/5) 95%CI 47.8-100%  <u>Negative</u> 99.0% (203/205) 95%CI 96.5-99.9%

\*Excluding 53 CDC IgG ELISA results (49 indeterminate and 4 equivocal samples).

† One of the presumptive positive samples was dengue PRNT positive and the other 4 presumptive positives were negative with WNV, dengue and SLE PRNT.

**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 sera that were 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 IgG ELISA was positive with 36.0% (27/75) of the 75 PRNT positives (calculating 4 equivocal as negative), equivocal with four samples, and negative with 44 samples.

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

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results				
	Neg	Eqv	Pos	Total	%
Serological sensitivity positive (WNV PRNT positive)	44	4	27	75	36.0% (27/75) 95% CI 25.2-92.3%

**Study Site 3: Focus Reactivity with West Nile IFA Negatives (n=157)**

A clinical laboratory located in the southwestern U.S. assessed reactivity with 157 retrospective West Nile IFA negative samples. The Focus IgG ELISA was 96.8% (152/157) negative with WNV IgG IFA negative samples (including two equivocal samples calculated as positive), and positive with three samples.

**Study Site 3: Focus Reactivity with West Nile IFA Negatives (n=157)**

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results				
	Neg	Eqv	Pos	Total	%
Negative agreement with presumptive WNV IFA negative	152	2	3	157	95.6% (152/157) 95% CI 91.1-98.2%

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

Focus assessed the device's reactivity with 50 sera from patients suspected of encephalitis/meningitis. A U.S. federal government laboratory provided the retrospective and masked sera. One sample was confirmed positive by West Nile 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 IgG ELISA was negative with 95.6% (47/49) of the WNV negative samples, and positive with the one positive confirmed by West Nile PRNT.

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

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results				
	Neg	Eqv	Pos	Total	%
Serological sensitivity (CDC IgG ELISA positive and WNV PRNT positive)	0	0	1	1	100% (1/1) 95% CI NA
Negative agreement with presumptive CDC IgG ELISA negative	47	0	2	49	95.9% (47/49) 95% CI 86.0-99.5%

**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 non-flavivirus tests (e.g., test for other infectious diseases). Positive samples were tested with a CDC West Nile IgG ELISA. The Focus West Nile ELISA IgG was negative with 96.8% (426/440) of the CDC ELISA negative samples (including 14 Focus equivocals calculated as positive), and was positive with 100% (21/21) of the CDC ELISA positive samples. Fifteen CDC ELISA IgG indeterminate samples were excluded from performance calculations.

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

Specimens Characterized by Reference Assays	Focus WNV IgG ELISA Results				
	Neg	Eqv	Pos	Total	%
Positive agreement with presumptive CDC IgG ELISA positive	0	0	21	21	100% (21/21) 95% CI 83.9-100%
Negative agreement with presumptive CDC IgG ELISA negative	426	14	0	440	96.8% (426/440) 95% CI 94.7-98.3%

\*Excludes 15 CDC IgG ELISA indeterminate samples.

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, 30.5% males, and 1.5% samples from persons of unspecified gender.

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

<b>Age</b>	<b>Neg</b>	<b>Eqv</b>	<b>Pos</b>	<b>% Positive</b>	<b>95%CI</b>
0 to 9	20	0	4	16.7% (4/24)	4.7-37.4%
10 to 19	25	2	2	6.9% (2/29)	0.9-22.8%
20 to 29	63	4	3	4.3% (3/70)	0.9-12.0%
30 to 39	76	1	5	6.1% (5/82)	2.0-13.7%
40 to 49	69	1	8	10.3% (8/78)	4.5-19.2%
50 to 59	47	1	3	5.9% (3/51)	1.2-16.2%
60 to 69	32	1	6	15.4% (6/39)	5.9-30.5%
70 to 79	28	1	5	14.7% (5/34)	5.0-31.1%
80+	15	1	2	11.1% (2/18)	1.4-34.7%
Unknown	41	2	8	15.7% (8/51)	7.0-28.6%
Overall	416	14	46	9.7% (46/476)	7.2-12.7%

**M. Conclusion:**

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