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FOCUS technologies

510(k) Summary of Safety and Effectiveness
West Nile Virus IgM Capture ELISA Catalog No. EL0300M
Prepared October 17, 2003

Applicant	Focus Technologies, Inc. 10703 Progress Way Cypress, California 90630 USA
Establishment Registration No.	2023365
Contact Person	Michael J. Wagner, Esq. tel (714) 220-1900 fax (714) 995-6921 mwagner@focustechnologies.com
Summary Date	October 17, 2003
Proprietary Name	West Nile Virus IgM Capture ELISA
Generic Name	West Nile Virus IgM Capture ELISA
Classification	West Nile Virus Serological Reagents 21 CFR §866.3940 Class II
Predicate Device	Focus Technologies Arbovirus IFA IgM (K913618) Focus Technologies HSV-2 ELISA (K993724) CDC West Nile Virus IgM Capture ELISA West Nile Virus Plaque Reduction Neutralization Test

Device Description

Indirect Enzyme-linked immunosorbent assay for qualitatively detecting human serum IgM antibodies to West Nile virus.

Intended Use

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 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.

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. Recombinant 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.

Expected Values

The prevalence of West Nile antibodies varies depending 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)

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%

Performance Characteristics

Study Site 1: Focus Reactivity with 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, 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	%	95% CI
Clinical sensitivity (encephalitis/meningitis symptoms, CDC WNV IgM ELISA positive and WNV PRNT positive)	2	2	40	44	90.9% (40/44)	78.3-97.5%
Positive agreement with presumptive CDC WNV IgM ELISA	0	0	2	2	100% (2/2)	15.8-100%
Negative agreement with presumptive CDC WNV IgM ELISA	251	2	1	254	98.8% (251/254)	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 samples 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	%	95% CI
Serological sensitivity (CDC WNV IgM ELISA positive and WNV PRNT positive)	0	0	75	75	100% (75/75)	95.2-100%

Performance Characteristics (continued)

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	%	95% CI
Negative agreement with presumptive WNV IFA	99	1	3	103	96.1% (99/103)	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 (LAC, EEE, SLE 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: Focus Reactivity with Suspected Encephalitis/Meningitis Patients (n= 50)

Specimens Characterized by Reference Assays	Focus WNV IgM ELISA Results					
	Neg	Eqv	Pos	Total	%	95% CI
Serological sensitivity (CDC WNV IgM ELISA positive and WNV PRNT positive)	0	0	1	1	100% (1/1)	NA
Negative agreement with presumptive CDC WNV IgM ELISA	48	0	1	49	98.0% (48/49)	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 non-flavivirus tests (e.g., tests for other 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 calculated 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	%	95% CI
Positive agreement with presumptive CDC WNV IgM ELISA	0	0	1	1	100% (1/1)	NA
Negative agreement with presumptive CDC WNV IgM ELISA	468	3	0	471	99.4% (468/471)	98.1-99.9%

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

Performance Characteristics (continued)

Focus Cross-reactivity

Focus (Study Site 4) 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 results of the studies are summarized in the table below.

Focus Cross-reactivity

Population	Site	Focus WNV IgM ELISA Results					
		Neg	Eqv	Pos	Total	% Positive	95% CI
Dengue virus (secondary infections)	4	14	1	5	15	40.0% (6/15)	16.3-67.7%
St. Louis encephalitis virus	1	6	0	7	13	53.8% (7/13)	25.1-80.8%
Eastern Equine Encephalitis virus	4	2	0	0	2	0.0% (0/2)	0.0-84.2%
Herpes simplex virus	4	18	1	1	20	10.0% (2/20)	1.2-31.7%
Epstein-Barr virus	4	19	0	0	19	0.0% (0/19)	0.0-17.6%
Cytomegalovirus	4	13	0	1	14	7.1% (1/14)	0.2-33.9%
Borrelia burgdorferi	4	0	0	3	20	15.0% (3/20)	3.2-37.9%
Rheumatoid factor	4	0	1	4	20	25.0% (5/20)	3.7-49.1%
Anti-nuclear antibodies	4	0	0	1	20	5.0% (1/20)	0.1-24.9%

Specificity of the Focus IgM Capture Wells

Focus (Study Site 4) assessed specificity of the IgM Capture Wells by selecting fifteen different sera that were positive for both WNV IgM and IgG, and treating the sera in four different ways:

1) No Treatment: The sera were not treated with DTT nor 2-ME, and the sera were diluted in the kit Sample Diluent (no goat anti-human IgG);

2) Goat anti-human IgG (GtαIgG): The sera were treated with diluent containing Goat anti-human-IgG;

3) Dithiothreitol (DTT): The sera were treated with 5 µL of 50 mM DTT and the sera were diluted in the kit Sample Diluent (no goat anti-human IgG);

4) 2-Mercaptoethanol (2-ME): The sera were treated with 5 µL of 1.43 M (10% v/v) 2-mercaptoethanol, and the sera were diluted in the kit Sample Diluent (no goat anti-human IgG).

All treatment groups were tested with the Focus WNV IgM ELISA, and the first two groups were tested with the Focus WNV IgG ELISA. The "No Treatment" groups showed that all 15 samples are clearly IgM and IgG positive, with indices ranging from 2.75 to 4.99. Treating with DTT caused 100% (15/15) of the samples to show at least a 50% decrease in reactivity, with two samples remaining positive, three samples becoming equivocal, and ten samples becoming negative. Treating with mercaptoethanol caused 100% (15/15) of the samples to become IgM negative. Treatment with goat anti-human IgG precipitating reagent caused 100% (14/14) of the samples to become IgG negative, while 100% (15/15) of the samples remained IgM positive.

Performance Characteristics (continued)

Sera Freeze-Thaw Study

Focus (Study Site 4) assessed the impact on the WNV IgM assay's reactivity by selecting 8 sera (5 positive and 3 negative), subjecting them to up to 5 repeated freeze-thaw cycles, and testing them in parallel with aliquots that had not been frozen. There were no changes in interpretation in any of the sera. Positive samples trended slightly towards increasing indices, while negative sera did not appear to change.

Focus 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.

Focus 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.

DEPARTMENT OF HEALTH & HUMAN SERVICES

Public Health Service

Food and Drug Administration
2098 Gaither Road
Rockville MD 20850

OCT 2 2 2003

Michael J. Wagner, Esq.
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Focus Technologies, Inc.
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Cypress, CA 90630

Re: k031952
Trade/Device Name: West Nile Virus IgM Capture ELISA
Regulation Number: 21 CFR 866.3940
Regulation Name: West Nile Virus Serological Reagents
Regulatory Class: Class II
Product Code: NOP
Dated: October 17, 2003
Received: October 20, 2003

Dear Mr. Wagner:

We have reviewed your Section 510(k) premarket notification of intent to market the device referenced above and have determined the device is substantially equivalent (for the indications for use stated in the enclosure) to legally marketed predicate devices marketed in interstate commerce prior to May 28, 1976, the enactment date of the Medical Device Amendments, or to devices that have been reclassified in accordance with the provisions of the Federal Food, Drug, and Cosmetic Act (Act) that do not require approval of a premarket approval application (PMA). You may, therefore, market the device, subject to the general controls provisions of the Act. The general controls provisions of the Act include requirements for annual registration, listing of devices, good manufacturing practice, labeling, and prohibitions against misbranding and adulteration.

If your device is classified (see above) into either class II (Special Controls) or class III (PMA), it may be subject to such additional controls. Existing major regulations affecting your device can be found in Title 21, Code of Federal Regulations (CFR), Parts 800 to 895. In addition, FDA may publish further announcements concerning your device in the Federal Register.

Please be advised that FDA's issuance of a substantial equivalence determination does not mean that FDA has made a determination that your device complies with other requirements of the Act or any Federal statutes and regulations administered by other Federal agencies. You must comply with all the Act's requirements, including, but not limited to: registration and listing (21 CFR Part 807); labeling (21 CFR Parts 801 and 809); and good manufacturing practice requirements as set forth in the quality systems (QS) regulation (21 CFR Part 820).

This letter will allow you to begin marketing your device as described in your Section 510(k) premarket notification. The FDA finding of substantial equivalence of your device to a legally marketed predicate device results in a classification for your device and thus, permits your device to proceed to the market.

If you desire specific information about the application of labeling requirements to your device, or questions on the promotion and advertising of your device, please contact the Office of In Vitro Diagnostic Device Evaluation and Safety at (301) 594-3084. Also, please note the regulation entitled, "Misbranding by reference to premarket notification" (21CFR Part 807.97). Other general information on your responsibilities under the Act may be obtained from the Division of Small Manufacturers, International and Consumer Assistance at its toll-free number (800) 638-2041 or (301) 443-6597 or at its Internet address http://www.fda.gov/cdrh/dsma/dsmamain.html.

Sincerely yours,

Steven Gutman

Steven I. Gutman, M.D., M.B.A.
Director
Office of *In Vitro* Diagnostic Device
Evaluation and Safety
Center for Devices and
Radiological Health

Enclosure

510(k) Number (if known): K031952

Device Name: West Nile Virus IgM Capture ELISA

Indications for Use: 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 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.

(PLEASE DO NOT WRITE BELOW THIS LINE CONTINUE ON ANOTHER PAGE IF NEEDED)

Concurrence of CDRH, Office of Device Evaluation (ODE)

Division Sign-Off 10/21/03

Office of In Vitro Diagnostic Device Evaluation and Safety

510(k) K031952

(Optional Format 3-10-98)

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Prescription use

OTC