

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

A. 510(k) Number:

K052519

B. Purpose for Submission:

This is an original application for a new rapid immunochromatographic assay for the qualitative determination of West Nile Virus IgM in human serum or plasma in patients having signs and symptoms of meningoencephalitis.

C. Measurand:

West Nile Virus IgM

D. Type of Test:

Rapid Immunochromatographic Assay

E. Applicant:

Spectral Diagnostics, Inc.

F. Proprietary and Established Names:

Spectral West Nile Virus IgM STATus™ Test

G. Regulatory Information:

1. Regulation section:

21 CFR 866.3940

2. Classification:

Class II

3. Product code:

NOP

4. Panel:

H. Intended Use:

1. Intended use(s):

The Spectral West Nile virus IgM STATus™ test is a rapid immunochromatographic lateral flow assay that utilizes recombinant West Nile virus (WNV) antigen (E glycoprotein) for the qualitative detection of IgM antibodies to WNV in human serum or plasma (sodium heparin or sodium citrate). This test is for the presumptive laboratory diagnosis of West Nile virus infection in patients having signs and symptoms of meningoencephalitis. Positive results must be confirmed by the PRNT (Plaque Neutralization Reduction Test), or by using the current CDC guidelines for diagnosis of this disease.

2. Indication(s) for use:

See Intended Use above

3. Special conditions for use statement(s):

This device is for prescription use only

4. Special instrument requirements:

Not Applicable

I. Device Description:

The Spectral WNV IgM STATus™ test employs solid-phase immunochromatographic assay technology to qualitatively detect the presence of WNV IgM antibodies in serum or plasma. See Test Principle below for more details.

J. Substantial Equivalence Information:

1. Predicate device name(s):

Focus West Nile Virus IgM Capture ELISA

2. Predicate 510(k) number(s):

K031952

3. Comparison with predicate:

Similarities		
Item	Device	Predicate
Antigen	Recombinant West Nile Virus antigen (E protein)	Recombinant West Nile Virus antigen (E protein)

Differences		
Item	Device	Predicate
Assay format	Rapid immunochromatographic	ELISA
Specimen types	Serum and plasma (sodium heparin or sodium citrate)	Serum only
Readout	Visible by operator	Optical Density by plate reader
Conjugate	Gold-labeled mouse anti-flavivirus	Mouse anti-flavivirus HRP

K. Standard/Guidance Document Referenced (if applicable):

Not Applicable

L. Test Principle:

The Spectral WNV IgM STATus™ test employs solid-phase immunochromatographic assay technology to qualitatively detect the presence of WNV IgM antibodies in serum or plasma. When the specimen to be tested is dispensed into the sample well of the Spectral device, anti-WNV IgM in the sample will bind to the recombinant WNV antigen (envelop glycoprotein (E) of West Nile virus, NY99 strain) to form a tertiary complex with gold-labeled monoclonal murine antibody against flavivirus family glycoprotein E. This tertiary complex will migrate through reaction strip and be captured by goat anti-human IgM antibodies at the Test area. Excess, unreacted gold complex detector is captured by immobilized anti-mouse IgG antibodies at the Control area. Assay results are determined by visual assessment of the presence or absence of control and test bands.

M. Performance Characteristics (if/when applicable):

1. Analytical performance:

a. *Precision/Reproducibility:*

Reproducibility studies were performed using 15 patient serum samples at 3 study sites over a three day period. Each day a new lot was used and a different operator was employed. The 15 member reproducibility panel consisted of 6 clinical specimens with a mean index value 25% above the cutoff, 6 clinical specimens with a mean index value 15% below the cutoff,

and 3 clinical specimens with a mean index value 6 times the cutoff level of a commercially available WNV IgM Capture ELISA device (comparator device). All sites and operators produced the expected result for all panel members on every day of testing.

b. Linearity/assay reportable range:

Not Applicable

c. Traceability, Stability, Expected values (controls, calibrators, or methods):

Not Applicable

d. Detection limit:

Not Applicable

e. Analytical specificity:

Interfering Substances

The following constituents of human blood were tested to ensure that they did not interfere with the performance of the Spectral device. The concentration of the serum proteins or lipids shown in Table below were added to positive and negative serum specimens and tested with the Spectral device. No interference up to the concentrations listed below was observed.

Potential Interferents and Concentrations

Potential Interfering material	Concentration
Human serum albumin	16 g/dL
Bilirubin (unconj.)	60 mg/dL
Hemoglobin	25 g/dL
Triglycerides	1.3 g/dL
Human IgM	300 mg/dL

IgM Specificity:

The IgM specificity of the Spectral WNV IgM test was assessed in 14 paired positive samples by treating with 5mM dithiothreitol (DTT) solution for one hr at room temperature (22-24°C). All 14 treated samples produced negative results following the treatment with DTT and their respective untreated paired samples remained positive, indicating the presence of IgM class antibodies in the positive samples (10).

Cross Reactivity:

Cross- reactivity studies with sera that were sero-positive to other potentially cross-reactive pathogens were conducted at a Public Health Laboratory in the North Eastern USA (site 1). Three out of seven Dengue post infection samples were tested at Public Health Laboratory at Mid-West Canada (site 3); 29 out of 33 RF positive sera and 23 out

of 28 ANA positive sera were tested in-house (site 2). Cross reactivity with Eastern Equine Encephalitis specimens was studied at a CDC laboratory at Mid-West USA (site 4). Results of these studies are tabulated below.

Results: Cross Reactivity Results

Potentially Cross-Reactive Agents	Spectral WNV IgM STATus™ test Results				
	Site	Number of Samples	Negative	Positive	% Cross-Reactive Samples
Herpes Simplex Virus 1 and/or 2	1	10	9	1	10%
Cytomegalovirus Polyvalent Test, all reactive	1	5	5	0	0%
Syphilis (trep Reactive)	1	10	10	0	0%
Epstein Barr Virus, IgG and IgM reactive	1	5	5	0	0%
ANA (anti-nuclear antibody)	1,2	28	17	11	39%
Rheumatoid Factor	1,2	33	23	10	30%
HIV(human immunodeficiency virus)	1	5	4	1	20%
Japanese Encephalitis Virus (vaccine recipients) IgM/IgG reactive,(CDC)	1	5	5	0	0%
Saint Louis Encephalitis Virus. SLE IgM (4/5) and IgG (5/5) reactive, CDC	1	5	5	0	0%
Dengue Virus (5 recent and 2 post infection)- DEN IgM & IgG reactive, CDC	1,3	10	9	1	10%
Hepatitis B Virus (HbsAg)	1	5	5	0	0%
Hepatitis C Virus (HCAb)	1	5	5	0	0%
California Encephalitis PRNT reactive as JC or LAX	1	6	6	0	0%
Legionella, Polyvalent Test, all reactive	1	5	5	0	0%
Yellow Fever (vaccine recipients)	1	5	4	1	20%
E. coli infection (culture confirmed)	2	4	4	0	0%
Eastern Equine Encephalitis	4	2	2	0	0%

f. Assay cut-off:

Not Applicable

2. Comparison studies:

a. *Method comparison with predicate device:*

Study sites 1-3: Negative agreement of Spectral device with comparator in non-flavivirus IgM samples:

Studies were performed at three sites on clinical serum specimens collected prospectively from patients with a variety of non-flavivirus ailments (rash, febrile, bronchitis, diarrhea, drug, neuropathy, acute coronary syndrome etc) and from endemic normal populations either at emergency departments of hospitals or at in-house collection. Site 1 represents an endemic region at the South-Western State of America; sites 2 and 3 represent endemic regions of South Eastern Provinces of Canada. These specimens were tested at individual sites concomitantly with the Spectral and a commercially available comparator device. The results are tabulated as “Pos” (positive), or “Neg” (negative). Indeterminate results produced by the comparator device are listed as equivocal (“Eqv”)

Study Site 1: Negative agreement of Spectral device with comparator in non-flavivirus IgM samples (n=160).

		Comparator device			Total
		Pos	Eqv	Neg	
Spectral Device	Pos	0	0	1**	1
	Neg	0	2*	157	159*
	Total	0	2	158	160

Negative agreement with the comparator device = $157/158^* = 99.4\%$ 96.5-99.9% (95% CI)

*Note: Two specimens that produced equivocal results with the comparator device were determined to have interference from crossreacting antibodies.

**One Spectral false positive result was observed and determined to be due to interference from crossreacting antibodies

Study Site 2: Negative agreement of Spectral device with comparator in non-flavivirus IgM samples (n=114).

		Comparator device			Total
		Pos	Eqv	Neg	
Spectral Device	Pos	0	0	2*	2
	Neg	0	-	112	112

	Total	0	0	114	114
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Negative agreement with the comparator device = 112/114 98.2% 93.8-99.8% (95% CI)

*Two Spectral false positive results were observed and determined to be due to interference from crossreacting antibodies

Study Site 3: Negative agreement of Spectral device with comparator in non-flavivirus IgM samples (n=72).

		Comparator device			Total
		Pos	Eqv	Neg	
Spectral Device	Pos	1*	0	1	2**
	Neg	0	0	70	70
	Total	1	0	71	72

Negative agreement with the comparator device = 70/71 98.6% 92.4-99.9% (95% CI)

*False positive due to cross reacting antibodies, as the sample remained positive with both devices when antigen was removed.

**Two Spectral false positive results were observed and determined to be due to interference from crossreacting antibodies.

All Sites (1-3): Negative agreement of Spectral device with comparator in non-flavivirus IgM samples (n= 346)

		Comparator device			Total
		Pos	Eqv	Neg	
Spectral Device	Pos	1*	0	4	5***
	Neg	0	2**	339	341*
	Total	1	2	343	346

Negative agreement with the comparator device = 339/343 98.8% 97.1-99.7% (95% CI)

*False positive due to cross reacting antibodies (e.g. RF & heterophiles), as the sample remained positive with both devices when antigen was removed.

**Two specimens that produced equivocal results with the comparator device were determined to have interference from crossreacting antibodies.

***Five Spectral false positive results were observed and determined to be due to interference from crossreacting antibodies

Study Site 4: A provincial health laboratory located at South Eastern Province of Canada assessed the performance of the Spectral device with randomized retrospective patient serum specimens (n=90) that were sent to the laboratory with suspected WNV infection based on clinical signs and symptoms. This batch of samples constituted 40 WNV PRNT confirmed and 50 CDC WNV IgM ELISA negative specimens. All specimens were randomized and tested blinded with the Spectral device.

Results Study Site 4: Spectral WNV IgM STATus™ test reactivity with WNV PRNT Positive/CDC WNV IgM ELISA positive and CDC WNV IgM ELISA negative specimens

Specimen Characterized by Reference Assays	Spectral WNV IgM STATus™ Results				
	Pos	Neg	Total	Pos or Neg Agreement	95%CI
Serological sensitivity (CDC WNV IgM and IgG ELISA Positive and WNV PRNT Positive), n=40	38	2	40	95%	83.1%-99.4%
Negative Agreement with presumptive CDC WNV IgM ELISA Negative, n=50	2	48	50	96%	86.3%-99.5%

Study Site 5:

Spectral device reactivity was assessed at a public health laboratory located in a prairie Province at Central Canada using a banked panel of clinical serum specimens that were drawn from patients (n=79) with clinical symptomology consistent with the West Nile virus infection. These samples also included symptomatic acutely infected patients (PRNT confirmed, n= 24) exhibiting either febrile illness or neuro-invasive disease such as meningitis or encephalitis and, others specimens from asymptomatic patients (n=25) that were sent to the laboratory to detect previous West Nile virus exposures. All these samples were then randomized, blinded and tested with the Spectral device.

Results Study Site 5: Spectral WNV IgM STATus™ test reactivity with WNV PRNT Positive/CDC WNV IgM Elisa positive and CDC WNV IgM Elisa negative specimens

Specimen Characterized by Reference Assays	Spectral WNV IgM STATus™ Results				
	Pos	Neg	Total	Pos or Neg Agreement	95%CI
Serological sensitivity for acute PRNT Pos, CDC WNV IgM & IgG Elisa Pos (n=24)	24	0	24	100%	88.3%-100%
Serological sensitivity for late PRNT Pos, CDC WNV IgM & IgG Elisa Pos (n=25)	20	5	25	80%	59.3%-93.2%
Negative agreement with presumptive CDC WNV IgM Elisa Neg (n=30)	1	29	30	97%	82.8%-99.9%

b. Matrix comparison:

In order to show that the Spectral device produces similar performance for all indicated matrices, 63 sodium citrate plasma and serum paired samples and 59 sodium heparin plasma and serum paired samples (obtained by spiking with the known WNV positive clinical specimens) were tested concomitantly with the Spectral device. Comparable results were obtained for sodium citrate plasma vs. serum and sodium heparin plasma vs. serum.

3. Clinical studies:

Not Applicable

4. Clinical cut-off:

Not Applicable

5. Expected values/Reference range:

Serum specimens from a total of 346 presumably healthy individuals (n=67) or non-WNV ailments (n=279) including 61 febrile patients were tested with the Spectral WNV IgM STATus™ test (henceforth referred to as the Spectral device) in a general population from endemic regions of USA and Canada. The samples were about equally distributed between male (47.1%) and female (52.9%) populations. Positive results were observed and determined to be positive due to heterophile antibodies interferences.

Prevalence of WNV IgM and Efficacy of Spectral Device in Endemic Population

Age (Years)	Negative	Positive	% Positive	95% CI
0 to 9	0	0	0.0% (0/0)	NA
10 to 19	8	0	0.0% (0/8)	0.00%, 31.23%
20 to 29	39	1	2.5% (1/40)	0.06%, 13.16%
30 to 39	56	1	1.7% (1/57)	0.04%, 9.39%
40 to 49	53	1	1.8% (1/54)	0.05%, 9.89%
50 to 59	56	0	0.0% (0/55)	0.00%, 5.30%
60 to 69	49	1	2.0% (1/50)	0.05%, 10.65%
70 to 79	39	1	2.5% (1/40)	0.06%, 13.16%
80+	41	0	0.0% (0/41)	0.00%, 7.05%
Overall	341	5	1.4% (5/346)	0.47%, 3.34%

N. Proposed Labeling:

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

O. Conclusion:

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