West Nile Virus IgM Capture DxSelect™ (English)

Enzyme-linked Immunosorbent Assay (ELISA)

Product Code EL0300M

Rev. M

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

For *in vitro* Diagnostic Use

FOCUS Diagnostics

INTENDED USE

The Focus Diagnostics West Nile Virus IgM Capture DxSelect™ is intended for qualitatively detecting IgM antibodies to West Nile virus in human serum. In conjunction with the Focus Diagnostics West Nile Virus IgG DxSelect™, 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 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.[1] 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.

Caution: IgM assay cross-reactivity has been noted with some West Nile IgM assays testing specimens containing antibody to enteroviruses. Reactive results reported from children must contain a caution statement regarding possible cross-reactivity with enteroviruses.

SUMMARY AND EXPLANATION OF TEST

Most people who are infected with West Nile virus (WNV) will not have any type of illness. Experts estimate that 20% of the people who become infected will develop West Nile fever: mild symptoms, including fever, headache, and body aches, occasionally with a skin rash on the trunk of the body and swollen lymph glands.[2] Symptoms of mild disease will generally last a few days. About 1 in 150 of West Nile virus infections (<1%) result in meningitis or encephalitis.[2] Case fatality rates among patients hospitalized during recent outbreaks have ranged from 4% to 14%.[3] Advanced age is the most important risk factor for death, and patients older than 70 years of age are at particularly high risk.[3] The case fatality rates for WNV are similar to St. Louis encephalitis (SLE) virus and Western equine encephalitis (WEE) virus (5-15%), but much lower than Eastern equine encephalitis (EEE) virus (30-70%), and higher than La Crosse (LAC) virus (<1%).[4,5]

WNV is an arbovirus. Arboviruses are zoonotic, and are transmitted through complex life cycles involving a vertebrate (e.g., birds) and an arthropod (e.g., mosquitoes).[3] Humans and domestic animals can develop clinical illness but usually are "dead-end" hosts because they do not produce significant viremia. Infection is usually not transmitted from person to person. Arbovirus infections can be prevented in two major ways: personal protective measures to reduce contact with mosquitoes and public health measures to reduce the population of infected mosquitoes in the environment.[2]

WNV can be detected by culturing the organism, by detecting viral antigen or by detecting viral nucleic acid in cerebrospinal fluid, tissue, blood, or other body fluid. Although a positive culture or a positive nucleic acid detection test are highly specific, experts do not recommend their use for screening because of their limited sensitivity.[2] Viral culture of cerebrospinal fluid or brain tissue has had very low yield among U.S. patients. Nucleic acid amplification testing has been positive in up to 55% of samples of cerebrospinal fluid and 10% of serum samples.[2] Centers for Disease Control and Prevention (CDC) recommends serology for detecting WNV and other arboviruses.[1]

CDC reports that although the antibody response to human infection with WNV has not been thoroughly or systematically studied, the following are reasonable assumptions, based on extensive experience with other flaviviruses, or preliminary conclusions based on empirical observations made during the 1999 and 2000 epidemics:

- IgM antibody in serum: By the eighth day of infection, a large majority of infected persons will have detectable serum IgM antibody to WNV; in most cases it will be detectable for at least 1-2 months after illness onset; and in some cases it will be detectable for 500 days or longer.[6]
- IgG antibody in serum: By three weeks post-infection (and often earlier), virtually all infected persons should demonstrate serum IgG antibody to WNV by enzymatic immunoassay (EIA) for 500 days or longer.[6]

Positive results are known to occur with persons vaccinated for flaviviruses (e.g., yellow fever, Japanese encephalitis, dengue), with persons infected with other flaviviruses, and with persons previously infected with WNV. Because closely related arboviruses exhibit serologic cross-reactivity, sometimes it may be epidemiologically important to attempt to pinpoint the infecting virus by conducting plaque reduction neutralization tests using an appropriate battery of closely related flaviviruses.

TEST PRINCIPLE

In the Focus Diagnostics West Nile Virus IgM Capture DxSelect™, the polystyrene microwells are coated with anti-human antibody specific for IgM (μ-chain). Diluted specimen 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 read by a spectrophotometer. The color intensity is compared to the Cut-off's to determine if antigen-specific IgM is present in the sample.

Background Subtract Procedure

All IgM reactive samples must be tested with the background subtract procedure to check for false positives caused by cross-reacting antibodies (e.g., RF and heterophilic antibodies) and other substances. Heterophile antibodies are antibodies that can be present in the patient specimen and can bind to animal antibodies (for example the Capture Wells contain rabbit antibody and the Anti-flavivirus Conjugate contains mouse antibody). The background subtract procedure detects false positives by testing initially positive samples with and without West Nile Antigen and comparing the reactivity. If heterophile antibodies are present in the sample, they will cross-link the Capture Well antibodies to the Anti-flavivirus Conjugate, and both wells will be reactive. If heterophile antibodies are absent, then only the well with Antigen will be reactive. The background subtraction method will not eliminate false positive results due to cross-reactive antibodies to other flaviviruses (e.g. St. Louis encephalitis, dengue etc).

MATERIALS SUPPLIED

The Focus Diagnostics West Nile IgM Capture DxSelect™ Test kit contains sufficient materials to perform 96 determinations. Allow the supplied reagents to be used to warm to room temperature before use. All un-opened materials are stable at 2 to 8°C until the expiration date stated on the reagent label.

Antigen (lyophilized) | REF | EL0322 | Ag | + |

2 vials containing lyophilized recombinant West Nile virus antigen. Each 8mL antigen vial will perform approximately 80 tests.

To reconstitute one vial of the antigen, add exactly 8mL of the Sample Diluent provided.

DO NOT USE DISTILLED WATER OR ANOTHER REAGENT OTHER THAN THE REAGENT PROVIDED FOR RECONSTITUTION. ASSAY RESULTS ARE INVALID IF ANY OTHER MATERIAL IS USED FOR RECONSTITUTION.

The lyophilized antigen appears as a "pearl" and it must be "tapped" to the bottom prior to opening and reconstituting. Allow the antigen to re-hydrate at room temperature for one hour prior to use: the antigen must be completely dissolved before use. Store the remaining antigen at 2 to 8°C for up to 60 days following reconstitution. **Avoid storing the reconstituted antigen at room temperature: remove from 2 to 8°C, withdraw the amount needed immediately, and return the unneeded antigen immediately to 2 to 8°C. Do not freeze.**

IgM Capture Wells, 96 wells | REF | EL1521 | Ab | IgM |

12 eight-well polystyrene break-apart microwell strips on a frame. Each well is coated with rabbit anti-human IgM. Each strip may be broken down into individual wells for cost effective use. To avoid condensation, allow the antigen strips to warm to room temperature before opening the sealed packets.

Anti-flavivirus Conjugate, 16 mL | REF | EL0302 | CONJ | IgM |

One vial of affinity-purified and peroxidase-conjugated mouse monoclonal anti-flavivirus. Contains protein, buffer, and non-azide preservatives. Ready to use.

Positive Control, 0.3 mL | REF | EL0315 | CONTROL | + |

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see **Specimen, Controls and Calibrator Preparation**, below).

Negative Control, 0.3 mL | REF | EL0312 | CONTROL | − |

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see **Specimen, Controls and Calibrator Preparation**, below).

Cut-Off Calibrator, 0.3 mL REF EL0303 CONTROL CAL

One vial of human serum. Contains 0.1% sodium azide as a preservative. Requires dilution before use (see **Specimen, Controls and Calibrator Preparation**, below).

Sample Diluent, 112 mL REF EL1608-112 DIL SPE

One vial of PBS containing protein, surfactant, and non-azide preservatives. Ready to use.

10X Wash Buffer, 100 mL REF EL0405 BUF WASH

One vial of surfactant in PBS with non-azide preservatives. Prepare a 1X wash buffer solution before use.

To prepare a 1X wash buffer solution, mix 100 mL 10X Wash Buffer with 900 mL distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved.

Substrate Reagent, 16 mL REF EL0009 SUBS TMB

One vial of tetramethylbenzidine (TMB) and hydrogen peroxide in buffer. A dark blue color indicates contamination with peroxidase; and if this occurs, then use a fresh bottle. Ready to use.

Stop Reagent, 16 mL REF EL0105 SOLN STOP

One vial 1 M sulfuric acid. Ready to use.

Sealing Tape

Three sheets of sealing tape.

MATERIALS REQUIRED, BUT NOT SUPPLIED

1. Distilled or deionized water
2. 250 or 500 mL wash bottle *or* automated EIA plate washer
3. 1 L graduated cylinder
4. 12 x 75 mm borosilicate glass test tubes or equivalent
5. 10 µL pipettor with disposable tips
6. 100 µL pipettor with disposable tips (100 µL eight-channel pipettor recommended for runs over 48 wells).
7. 1 mL pipet or dispenser
8. 5 mL pipet
9. Timer
10. Paper towels or absorbant paper
11. Sink
12. Vortex mixer or equivalent
13. ELISA plate spectrophotometer, wavelength = 450 nm

SHELF LIFE AND HANDLING

1. Kits and kit reagents are stable through the end of the month indicated by the label expiration date when stored at 2 to 8°C.
2. Do not use test kit or reagents beyond their expiration dates.
3. Do not expose reagents to strong light during storage or incubation.
4. Allow reagents to warm to room temperature before use.

WARNINGS AND PRECAUTIONS

1. This kit is for *in vitro* diagnostic use only.
2. All blood products should be treated as potentially infectious. Source materials from which this product (including controls) was derived have been screened for Hepatitis B surface antigen, Hepatitis C antigen and HIV-1/2 (AIDS) antibody by FDA-approved methods and found to be negative. However, as no known test methods can offer 100% assurance that products derived from human blood will not transmit these or other infectious agents, all controls, serum specimens and equipment coming into contact with these specimens should be considered potentially infectious and decontaminated or disposed of with proper biohazard precautions. Centers for Disease Control and Prevention and the National Institutes of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.[7]
3. The Capture Wells are produced with anti-human IgM antibodies. After adding patient or control specimens, the strips should be considered potentially infectious and handled accordingly.
4. Sodium azide at a concentration of 0.1% has been added to some reagents as an antibacterial agent. To prevent buildup of explosive metal azides in lead and copper plumbing, those reagents (see Materials Supplied, above) should be discarded into sewerage only if diluted and flushed with large volumes of water. Use copper-free and lead-free drain systems where possible. Occasionally decontaminate the drains with 10% sodium hydroxide (CAUTION: caustic), allow to stand for 10 minutes, then flush with large volumes of water.
5. Do not substitute or mix reagents from different kit lots or from other manufacturers.

6. Use only protocols described in this insert. Incubation times or temperatures other than those specified may give erroneous results.
7. Cross-contamination of patient specimens can cause erroneous results. Add patient specimens and handle strips carefully to avoid mixing of sera from adjoining incubation tray wells. Decant carefully.
8. Bacterial contamination of serum specimens or reagents can produce erroneous results. Use aseptic techniques to avoid microbial contamination.
9. The Stop Reagent contains sulfuric acid. Do not allow to contact skin or eyes. If exposed, flush with copious amounts of water.
10. Sodium azide inhibits conjugate activity. Clean pipette tips must be used for the conjugate addition so that sodium azide is not carried over from other reagents.

SPECIMEN COLLECTION AND PREPARATION

This test must be performed on serum only. The use of whole blood, plasma or other specimen matrix has not been established. Performance characteristics have not been established with hyper-lipemic, heat inactivated, hemolyzed, icteric, or contaminated sera. It is unknown if such specimens will cause erroneous results. Hyper-lipemic, heat inactivated, hemolyzed, icteric, and contaminated sera must not be tested.

Specimen Collection and Handling

Collect blood samples aseptically using approved venipuncture techniques by qualified personnel. Allow blood samples to clot at room temperature prior to centrifugation. Aseptically transfer serum to a tightly closing sterile container for storage. Separated serum should remain at 22°C for no longer than 8 hours. If the assay will not be completed within 8 hours, refrigerate the sample at 2 to 8°C. If the assay will not be completed within 48 hours, or for shipment of samples, freeze at –20°C or colder. [8] Thaw and mix samples well prior to use.

Serum Specimen, Controls and Calibrator Preparation

Dilute each serum specimen, serum control and calibrator 1:101. For example, label tubes and dispense 1000 µL of Sample Diluent into each labeled tube. Add 10 µL of specimen, control or calibrator to each appropriate tube containing the 1000 µL Sample Diluent and mix well by vortex mixing.

TEST PROCEDURE

Perform the assay at room temperature (approximate range 20 to 25°C). Use proper pipetting techniques, maintaining the pipetting pattern throughout the procedure to ensure optimal and reproducible values. Performance characteristics have not been established for procedures that are different from the procedure described below. Different procedures, e.g., different times, volumes, temperatures, or others, may produce invalid results.

1. Allow all reagents to warm to room temperature before use. Remove the **IgM Capture Well** packet from cold storage. To avoid condensation, allow micro-well strips to reach room temperature before opening the foil packet. If less than a full plate is to be used, return unused strips to the foil packet with desiccant and reseal completely. Store unused **IgM Capture Wells** at 2 to 8°C. (**Note:** At the end of the assay, retain the frame for use with the remaining strips.)
2. Prepare Antigen Solution (make sure reagent has reached room temperature). If the kit is being used for the first time, reconstitute sufficient antigen (see **Materials Supplied**, above).
3. Fill wells with **1X Wash Buffer solution** (see **Materials Supplied**, above) and allow to soak for 5 minutes. Decant (or aspirate) the **IgM Capture Wells** and tap vigorously to remove Wash Buffer. Blot the emptied Capture Wells face down on clean paper towels or absorbant paper to remove residual Wash Buffer.
4. Dispense 100 µL of the **Sample Diluent** into the "blank" well(s) and 100 µL of each **diluted specimen, control or calibrator** (see **SPECIMEN COLLECTION AND PREPARATION**, above) into the appropriate wells. (**Note:** For runs with more than 48 wells it is recommended that 250 µL of each diluted sample first be added to a blank microtiter plate in the location corresponding to that in the ELISA wells. The samples can then be efficiently transferred into the Antigen Wells with a 100 µL 8 or 12-channel pipettor.)
5. Cover plates with sealing tape, and incubate for 60 ± 1 minute at room temperature (20 to 25°C).
6. Remove sealing tape, and empty the contents of the wells into a sink or a discard basin.
7. Fill each well (250 µL) with a gentle stream of **1X Wash Buffer** solution then empty contents into a sink or a discard container.
8. Repeat wash (step 7) an additional 2 times, allow the last wash to soak for five minutes before decanting or aspirating.
9. Tap the Capture Wells vigorously to remove 1X Wash Buffer. Blot the emptied Capture Wells face down on clean paper towels or absorbant paper to remove residual 1X Wash Buffer.
10. Add the prepared (see Step 2, above) **100 µL Antigen Solution** to all wells, using a 100 µL 8 or 12-channel pipettor.
11. Cover plates with sealing tape and incubate for 2 hours at room temperature (20 to 25°C).
12. Repeat wash steps 6 through 9.
13. Add 100 µL of **IgM Conjugate** to all wells, using a 100 µL 8 or 12-channel pipettor.
14. Cover plates with sealing tape, and incubate for 30 ± 1 minute at room temperature (20 to 25°C).
15. Repeat wash steps 6 through 9.

16. Add 100 µL of **Substrate Reagent** to all wells, using a 100 µL 8 or 12-channel pipettor. Begin incubation timing with the addition of Substrate Reagent to the first well. (**Note**: Never pour the substrate reagent into the same trough as was used for the conjugate.)
17. Incubate for 10 ± 1 minutes at room temperature (20 to 25°C).
18. Stop the reaction by adding 100 µL of **Stop Reagent** to all wells using a 100 µL 8 or 12-channel pipettor. Add the Stop Reagent in the same sequence and at the same pace as the Substrate was added. In antibody-positive wells, color should change from blue to yellow.
19. Gently blot the outside bottom of wells with a paper towel to remove droplets that may interfere with reading by the spectrophotometer. Do not rub with the paper towel as it may scratch the optical surface of the well. (**Note**: Large bubbles on the surface of the liquid may affect the OD readings.)
20. Measure the absorbance of each well within 1 hour of stopping the assay. Set the microwell spectrophotometer at a wavelength of 450 nm. Zero the instrument on the blank wells.

IgM Procedure (condensed version)

1. Dilute samples
   Serum samples and Controls: 1:101 in Sample Diluent. (e.g., 10 µL + 1000 µL)
2. Soak Wells for **5 minutes** with **1X Wash**, decant.
3. **100 µL** of **sample** for **60 minutes**, decant.
   Background subtract only: 100 µL of diluted sample is added to each of two wells. One well (the "Ag" well, "Ag" for antigen) gets WNV antigen in Step 5, and the other well (the "SD" well, "SD" for diluent) gets Sample Diluent in Step 5. **Important note:** Background subtract is one way to check for heterophile antibodies present in positive samples. Therefore, background subtract should not be performed unless the patient sample was initially positive.
4. Wash 3 times.
5. **100 µL** of **Antigen** for **120 minutes**, decant.
   Background subtract only: Add 100 µL of Antigen to the "Ag" well, and add 100 µL Sample Diluent to the "SD" well, incubate for 120 minutes.
6. Wash 3 times.
7. **100 µL** of **Conjugate** for **30 minutes**, decant.
8. Wash 3 times.
9. **100 µL** of **Substrate Reagent** for **10 minutes**.
10. **100 µL** of **Stop Reagent**, read at λ = 450 nm.

Please see the **PROCEDURE** section for important details.

QUALITY CONTROL

Each plate run (or strips or wells from a single plate) must include the Cut-off Calibrator and the two Controls. If multiple plates are run, include the Cut-off Calibrator and both controls on each plate. It is recommended that until the user becomes familiar with the kit performance, all specimens, controls and the Cut-off Calibrator should be run in duplicate with the Cut-off Calibrator run twice for a total of four wells. If single wells are used, the Cut-off Calibrator should be run in triplicate. Include a minimum of 1 blank well (containing sample diluent only) for instrument calibration purposes.

The Cut-off Calibrator has been formulated to give the optimum differentiation between negative and positive sera. Although the absorbance value may vary between runs and between laboratories, the mean value for the Cut-off Calibrator wells must be within 0.100 to 0.700 OD units. All replicate Cut-off Calibrator ODs should be within 0.10 absorbance units from the mean value. Report results as index values relative to the Cut-off Calibrator. To calculate index values, divide specimen and control optical density (OD) values by the mean of the Cut-off Calibrator OD values.

Calculation for the Background Subtract Results

Step 1. Calculate the **Index for the Controls** by dividing the Control OD by the Cut-off OD.

Index for the Controls (Cut-off and Control Index) = "Ag" OD/CO OD

Step 2. Calculate the **Index for Patient Samples** by first calculating the Net Patient OD (by subtracting the "SD" OD from the "Ag" OD), and then dividing the Net Patient OD by the Cut-off OD.

Net Patient OD = "Ag" OD - "SD" OD

Index for Patient Samples = Net Patient OD/CO OD

Step 3. Interpret using the ranges in the Interpretation section (e.g., negative < 0.90, and positive > 1.10).

Example Calculation for Background Subtraction Results

ID	OD*		Step 1	Step 2		Step 3
	Ag OD	SD OD	Divide Control OD by Cut-off OD **Ag OD /CO OD = Index for Controls**	Subtract SD OD from Ag OD **Ag OD – SD OD = Net OD for Patients**	Divide the Net OD by the Cut-off OD **Net OD/CO OD = Index for Patients**	**Interpretation**
Neg Control	0.008		0.02			
Cut-off	0.400		1.00			
Pos Control	1.200		3.00			
Specimen A	0.860	0.020		0.840	2.10	POS
Specimen B	0.890	0.010		0.880	2.20	POS
Specimen C	0.760	**0.720**		0.040	**0.10**	NEG

* Blank OD is already subtracted from each result.

Discussion of Background Subtraction Example Calculations

Specimen A and B should be interpreted as IgM positive because the index after subtracting the background is still greater than 1.10. (the wells with no antigen was not reactive).

Specimen C demonstrates the importance of subtracting background. Sample C was reactive even when Sample Diluent was added instead of West Nile antigen, indicating that antibodies in the sample were cross-linking the IgM Capture antibodies and the monoclonal conjugate. Specimen C should be considered IgM negative because the index value after subtracting background was 0.10, and 0.10 is less than 0.90 (thus in the negative interpretation zone).

1. The Positive Control index values must be between 1.5 and 3.5.
2. The Negative Control index values must be less than 0.8.

If the Calibrator or Controls are not within these parameters, patient test results must be considered invalid and the assay repeated. Invalid test results must not be reported.

The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control should not be used as an indicator for Cut-off Calibrator precision and only ensures reagent functionality. Quality control requirements must be performed in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's standard Quality Control procedures. In the US, regulatory authorities recommend that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices.

INTERPRETATION OF TEST RESULTS

To calculate index values, divide specimen optical density (OD) values by the mean of the Cut-off Calibrator absorbance values. The magnitude of the index value above the Cut-off Calibrator does not indicate the total amount of antibody present.

Cut-off Development. 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 four different serum panels (run without the background subtract method): 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 native antigen IgM ELISA positive[9,10]); 3) 108 presumed negative samples (US Public Health IgM ELISA and/or in-house WNV native antigen IgM ELISA negative); and 4) 7 ELISA discrepants (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.

IgM Index	Interpretation (IgM Result Only)
> 1.10	**IgM Positive.** An index value of > 1.10 indicates IgM antibodies to West Nile virus were detected. The presence of IgM antibodies is presumptive evidence that the patient was recently or is currently infected with West Nile virus or another flavivirus. Positive results must be tested using the background subtraction method (either on the initial test or on a repeat test). A patient can be IgM positive but not currently infected with West Nile virus because of 1) cross-reactivity to other flaviviruses[2] or 2) IgM antibodies from previous infections may be present for over 500 days.[2] IgM Positive results reported for children must contain a caution statement regarding possible cross-reactivity with enteroviruses. IgM positive results must be confirmed by plaque reduction neutralization test, or by using the recent CDC guidelines for diagnosis of West Nile encephalitis.
≤ 1.10 and ≥ 0.90	**IgM Equivocal.** An index value of ≥ 0.90 but ≤ 1.10 is considered an equivocal result. It is recommended that samples with equivocal results be tested using a different method, or the patient may be re-drawn two or more weeks later and re-tested with this assay.
< 0.90	**IgM Negative.** An index value of < 0.90 indicates IgM antibodies to West Nile virus were not detected. The absence of IgM antibodies is presumptive evidence that the patient was not recently infected with West Nile virus or another flavivirus. However, the sample may have been drawn before antibodies were detectable, or the patient may be immunosuppressed. If infection is suspected, then another sample should be drawn 7 to 14 days later and tested. Refer to CDC guidelines for diagnosis of West Nile encephalitis.

If the Focus West Nile ELISA IgG results are also available for the same specimen then use the following interpretation:

IgM Result	IgG Result	Combined Interpretation (Both IgM & IgG Results)
Pos	Pos	**Presume the patient was recently infected with WNV.** The presence of IgM antibodies is presumptive evidence that the patient was recently or is currently infected with West Nile virus or another flavivirus. However, IgM anti-WNV has been shown to persist for ≥ 500 days.
	Neg	**Refer to results above for IgM anti-WNV reactive results.**
Neg	Pos	**Presume the patient was previously infected with (or exposed to) WNV.** The presence of IgG antibodies without IgM antibodies is presumptive evidence that the patient was infected with (or exposed to) West Nile virus or another flavivirus at an undetermined time.
	Neg	**Presume the patient has not been infected (or exposed to) with WNV.** The absence of IgM and IgG antibodies is presumptive evidence that the patient has not been recently infected with (or exposed to) West Nile virus or another flavivirus.

LIMITATIONS

1. The performance of this assay has not been established for screening the general population. Testing should only be performed on patients with clinical symptoms of meningioencephalitis.
2. The performance of this assay has not been established for ruling out diseases with similar symptoms, e.g., herpes simplex virus encephalitis, enterovirus encephalitis, bacterial meningitis, causes of non-infectious encephalitis, or post-infectious encephalitis.
3. The performance of this assay has not been established for matrices other than serum, or visual result determination(s), or monitoring West Nile virus therapy.
4. All results from this and other serologies must be correlated with clinical history, epidemiological data, and other data available to the attending physician in evaluating the patient. Positive results must be confirmed by neutralization test, or by using the current CDC guidelines for diagnosing West Nile encephalitis.[1]
5. The prevalence of infection will affect the assay's predictive value.
6. This assay may cross-react with antibodies produced to other flaviviruses (e.g., dengue virus, Japanese encephalitis virus, St. Louis encephalitis virus, yellow fever virus, and others). These diseases must be excluded before confirmation of diagnosis.

EXPECTED VALUES

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.[11] 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.[2]

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%

PERFORMANCE CHARACTERISTICS

Performance characteristics without background subtract are in the left column, and with background subtract is in the right column.

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.[12] 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).[12] 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). 4 of the 256 presumptive assay results showed NS and were excluded.

Without Background Subtract
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 252 patients with presumptive assay results, 250 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 99.6% (249/250) of the presumed negative patients (including 1 Focus equivocal calculated as positive).

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

With Background Subtract
The Focus IgM assay was positive with 93.2% (41/44) of the confirmed positive WNV encephalitis patients (including 1 Focus equivocal calculated as negatives). Of the 252 patients with presumptive assay results, 250 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 100% (250/250) of the presumed negative patients.

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	1	41	44	93.2% (41/44) 95%CI 81.3-98.6%
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 & Study Site 4: Focus Reactivity with WNV PRNT Positives (n = 75)
Focus (background subtract) and a clinical laboratory (screening procedure) 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[9,10], 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.

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%

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 = 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.[13]

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).

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%

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	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 (LAC, EEE, 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)

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.

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%
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.

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	469	0	0	469	100% (469/469) 95% CI 99.2-100%

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

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 tables below:

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	60.0% (9/15) 95%CI 32.3-83.7%
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	7	0	1	8	12.5% (1/8) 95%CI 0.3-52.7%
Rheumatoid factor	4	15	1	4	20	25.0% (5/20) 95%CI 3.7-49.1%
Anti-nuclear antibodies	4	19	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%

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	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	14	0	0	14	0.0% (0/14) 95%CI 0.0-23.2%
Borrelia burgdorferi	4	8	0	0	8	0.0% (0/8) 95%CI: 0.0-36.9%
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.

Focus Reproducibility

Focus (Study Site 4), a clinical laboratory located in the mid-west United States (Study Site 5), and university laboratory located in northern California (Study Site 6) 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

Specificity of the Focus WNV IgM Assay

Focus (Study Site 4) assessed specificity of the WNV IgM Assay by selecting fifteen different sera that were positive for both WNV IgM and IgG. The sera were treated with 5 µL of 1.43 M (10% v/v) 2-mercaptoethanol (2-ME). Treating with 2-ME caused 100% (15/15) of the samples to become IgM negative.

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.

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), a clinical laboratory located in the mid-western U.S. (Study Site 2), and 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.

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.

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