WEST NILE (FLAVIVIRUS) IgM

I. Introduction

The Focus Technologies Flavivirus (West Nile) IgM capture ELISA is intended for qualitatively detecting IgM antibodies to flaviviruses(West Nile) in human serum. In conjunction with the Focus Technologies Flavivirus (West Nile) ELISA IgG, the test is indicated for testing persons having symptoms of arbovirus infection, as an aid in the presumptive diagnosis of flavivirus infection.

II. Specimen Collection and Processing

5 ml of blood is collected in a serum separator tube and separated by centrifugation. The serum is removed from the clot and refrigerated until testing. Specimens are stored at -70^0C after testing.

III. Procedure

i) Reagent Preparation:

a. Wash Buffer (100 ml) 10X
   Prepare working buffer (1X) by adding 100 ml of 10X Wash Buffer to 900 ml of Distilled H2O. Mix well. Labeled and dated.
b. West Nile Antigen (2 vials)
   Add 8 ml of the Sample Diluent to 1 vial of Antigen.(Do not use Distilled H2O). Keep re-constituted Antigen in fridge until use, and return it immediately to fridge after use. Each re- constituted Antigen can perform aproximately 80 tests and expired in 14 days..
c. Controls: 1 vial of Positive Control
   1 vial of Negative Control
d. 1 vial of Cut-off Calibrator
e. IgM Conjugate
f. Substrate Reagent
   2 vials of Tetramethylbenzidine (TMB) and Hydrogen Peroxide in buffer
g. Stop Reagent
   1 vial of 1M sulfuric acid

ii) Other Materials:
IgM Capture Wells, 96 wells
Sample Diluent

iii) Method:

1. Label a set of 12x75 mm glass tubes as follows: Blk, Pos,Neg, Cal,7,8,9,10….,and External Control (when required, e.g. a new lot #).
2. Add 1 ml of Sample Diluent to all tubes.
3. Leave 1st tube blank; add 10 ul of Controls, Calibrator and patient samples to corresponding tubes.
4. Take out the required # of strips (Open bag containing strips when strips reaches room temperature, ~10-15 minutes). Return the unused strips to the bag, and refrigerate immediately.
5. Change bottle from D H2O to Wash Buffer.

a. Turn Washer 'ON'.
b. Press button under 'Prime' (F2).
c. Press button under 'washing' (F1).
d. 'Select Program': Press memory (F1), press Number (F1).
e. **For dispensing 350 ul of buffer use Program '2'**.
f. 'Program No : press '2',enter.
g. '002 Name 1'should appear.
h. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is What is needed. If it is the same, press 'Escape'. If is not the same, enter the right # of strip, and press 'enter'until '002 Name 1' appears, press 'Enter'.
i. Press' Run' (F1), and press 'No' (F2) under 'Prime'.
j. The Washer will dispense the 350 ml of buffer into each well, and soak for 5 minutes.

6. After 5 minutes. Aspirate Wash Buffer from wells.
For aspiration of Wash Buffer, use Program ' 1 '.
   a. 'Select Program': Press memory (F1), press Number (F1).
   a. 'Program No: press 1,enter.
   b. '001 Name 01'should appear.
   c. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'Number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, press 'enter'. If is not the same, enter the right # of strip, and press 'enter 'until ' 001 Name 01'appears, press 'Enter'.
   d. Press' Run' (F1), and press 'No'(F2) under 'Prime'.
   e. The Washer will aspirate the buffer.

f. 'End washing' will appear when washing cycle is finished, remove plate.
g. 'Repeat Washing?' will appear, press 'No' (F2).

7. Add 100 ul of Blk to well # 1, 100 ul of Pos to well # 2, 100 ul of Neg to well # 3, 100 ul each of Cal to well # 4, 5, and 6. Add 100 ul of specimen to each well, starting from well # 7. Use last well as external Control well when needed.
8. Cover plate with sealing tape, and incubate for 60 minutes at Room Temperature.
9. Wash the wells three times using the Automatic Washer under Program '11'.

h. 'Select Program': Press memory (F1), press Number(F1).
i. For washing and soaking, use Program ' 11 '.
j. 'Program No : press 11,enter.
k. Program '11 Name IgM WN' should appear. Press 'Set' at this point, press 'Change' (F2), then press 'Enter' until 'number of strips to wash' appears-check that # of strip appears is what is needed. If it is the same, presses 'enter'. If is not the same, enter the right # of strip, and press 'enter' until '11 Name IgM WN' appears, press 'Enter'.
m..Press 'Run' (F1), and press 'No'(F1) under 'Prime'. Will wash strip for 3 times, each time with working buffer.
n.'End washing' will appear when washing cycle is finished, soak strip for 5 minutes.
o. Aspirate Wash Buffer using Program '1',steps 6 a-i.

10. Remove plate from Plate Washer.
11. Add 100 ul of Antigen into each well using an 8-channel pipette, cover plate with sealing tape and incubate for 2 hours (120 minutes) at room temperature.
12. Repeat wash steps 9a-h.
13. Add 100 ul of Conjugate into each well using an 8-channel pipette, cover plate with sealing tape and incubate for 30 minutes at room temperature.
14. Repeat wash steps.
15. Add 100 ul of Substrate into each well using an 8-channel pipette, cover and incubate for 10 minutes at room temperature.
16. Add 100 ul of Stop Reagent into each well using an 8-channel pipette. In antibody-positive wells, color should change from blue to yellow.
17. Read the plate in spectrophotometer at dual wavelength 450 nm/630 nm within 1 hour of stopping the assay.

iv) Calculation:

Index value= Optical Density of Sample/the mean Optical Density of the Cut-Off Calibrators

v) Interpretation of Index Values:

Positive: > 1.10

Negative: < 0.9

Equivocal: >=0.9 and <= 1.1

All positive and equivocal specimens should be repeated the next day .

IV. Validation

The mean value for the Cut-Off Calibrator must be within 0.100 to 0.700 O.D. units minus the blank well.

The Positive Control Index value should be between 1.5 and 3.5.

The Negative Control Index value should be less than 0.8.

V. Reporting

Positive result: To PHL Report PHL result in LIS, and inform physician if result is confirmed Positive.

Negative result: West Nile Virus (Flavivirus) IgM by EIA: Negative.
This is a research test.

Equivocal result: repeat testing,
If result remains equivocal/ becomes Positive: Send to PHL.
Inform physician if result from PHL is Positive.
If result becomes negative : West Nile Virus(Flavivirus) IgM by EIA: Negative.

VI. Quality Control

Each plate must include 1 Positive Control, 1 Negative Control, and 3 Cut-off Calibrators.

An External Positive Control (Accurun 165 West Nile virus IgM/IgG Positive Control Series 5000)must be included with each new lot. Result filed in Reagent Lot Binder.

Inform charge/senior technologist of any QC failure.

VII. Reference

Manufacture's insert, Focus Technologies, Cypress, California 90630.U.S.A. 2003.