DRG® West Nile IgG ELISA (EIA-4519)

Revised 28 May 2009 (Vers. 3.0) **USA: RUO**

1 INTENDED USE

The West Nile IgG Test for exposure to West Nile Virus (WNV) is an ELISA assay system for the detection of antibodies in human serum to WNV derived recombinant antigen (WNRA) (1-3). This test is to aid in the diagnosis of human exposure to the West Nile Virus.

It is not intended to screen blood or blood components.

In the United States, this kit is intended for Research Use Only.

2 SUMMARY AND EXPLANATION OF THE TEST

Exposure to West Nile Virus causes a disease with a number of symptoms including encephalitis (4-7). West Nile Virus is becoming widespread and has been detected in over half of the 50 states. This test has been developed and refined using reagents produced by CDC. The West Nile assay employs a recombinant antigen called WNRA, which can be used as a rapid serological marker for WNV infection. The WNRA protein is a recombinant antigen, which consists of a stretch of peptides from two WNV antigens.

3 PRINCIPLE OF THE TEST

The West Nile IgG ELISA consists of two enzymatically amplified "two-step" sandwich-type immunoassays.

In this assay, the microtitration wells are incubated with standards, controls or unknown serum samples. The serum samples may be directly mixed with sample dilution buffer added in the wells (also see note below). After washing, the wells are treated with an antibody specific for human IgG and labeled with the enzyme horseradish peroxidase (HRP). After a second incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate.

Note: Depending on the strength of antibody response, sera can be diluted in a diluent provided in the kit.

An acidic stopping solution is then added and the degree of enzymatic turnover of the substrate is determined by absorbance measurement at 450 nanometers. Above a certain threshold, the ratio of the absorbances of the WNRA and the control wells accurately determines whether antibodies to WNV are present. A set of positive and negative samples is provided as internal controls in order to monitor the integrity of the kit components.

4 MATERIALS SUPPLIED

The West Nile IgG ELISA contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each. Each kit contains the following reagents:

IgG Assay Specific Materials:

1. Ready-to-use Coated WN IgG **Microtiter Strips**
   Strip holder with plastic cover, containing 96 polystyrene microtiter wells (12 x 8 wells) coated with monoclonal antibody bound to recombinant WN antigen.
   Store at 2-8ºC until ready to use.
   Note: The WNRA and NCA are already bound to plates.
2. **Sample Dilution Buffer** For IgG,
   One bottle, 25 ml, for serum/plasma sample dilution.
   Store at 2-8ºC until ready to use.

3. WN IgG **Positive Control** (IgG P)
   One vial, 50 µL of positive serum to be used as a positive control.
   The positive control will aid in monitoring the integrity of the kit as well.
   Store at – 70°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
   Note: For long-term storage, serum can be further aliquoted in a smaller volume and stored at -70C.
4. WN IgG **Negative Control** (IgG N)
   One vial, 50 µL of heat–inactivated negative serum to be used as a negative control.
   The negative control will aid in monitoring the integrity of the kit as well.
   Store at – 70°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
   Note: For long-term storage, serum can be further aliquoted in a smaller volume and stored at -70C.
5. Ready to Use **Enzyme Conjugate-HRP** For WN IgG
   One bottle, 6 mL of a pre-diluted goat anti-human IgG conjugate to be used as is in the procedure below.
   Store at 2-8°C until ready to use.
6. **10X Wash Buffer**
   One bottle, 120 mL of Wash Buffer to be used in all the washing steps of this procedure.
   Store 10X Wash Buffer at 2-8°C until ready to use.
7. **EnWash**
   One bottle, 20 mL of EnWash to be used in between the washing steps after the addition of enzyme conjugate-HRP of this procedure.
   Store EnWash Buffer at 2-8°C until ready to use.
8. **TMB Substrate**
   One bottle, 9 mL of liquid substrate to be used in this procedure.
   Store at 2-8ºC until ready to use.
   The substrate should be kept in a light -protected bottle at all times.
9. **Stop Solution**
   One bottle, 6 mL to be used to stop the reaction.
   Store at 2-8°C until ready to use.

Caution: strong acid–wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

NOTE:
All reagents and controls must be allowed to reach room temperature (~25°C) and mixed thoroughly by gentle inversion prior to use. Always practice sterile and aseptic techniques at every step. For example, open all reagents in a sterile hood to avoid contamination with airborne bacteria to maintain shelf life.

5 MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- Vacuum Pump
- Humidified Incubator or Water Bath
- Single-Channel and Multi-Channel Pipetters

6 PRECAUTIONS

- A thorough understanding of this package insert is necessary for successful use of the product. Reliable results will only be obtained by using precise laboratory techniques and accurately following the package insert.
- Do not mix various lots of any kit component within an individual assay.
- Do not use any component beyond the expiration date shown on its label.
- Avoid exposure of the reagents to excessive heat or direct sunlight during storage and incubation.
- Some reagents may form a slight precipitate, mix gently before use.
- Incomplete washing will adversely affect the outcome and assay precision.
- To minimize potential assay drift due to variation in the substrate incubation time, care should be taken to add the stopping solution into the wells in the same order and speed used to add the TMB solution.
- Avoid microbial contamination of reagents, especially of the conjugate concentrate and the conjugate diluent. Avoid contamination of the TMB Substrate Solution with the Enzyme Conjugate-HRP.
- Wear protective clothing, eye protection and disposable gloves while performing the assay. Wash hands thoroughly afterwards.
- Do not eat, drink, smoke or apply cosmetics where immunodiagnostic materials are being handled.
- Do not pipet by mouth.
- Use a clean disposable pipette tip for each reagent, Standard, Control or specimen.
- Cover working area with disposable absorbent paper.

WARNING: POTENTIAL BIOHAZARDOUS MATERIAL
This kit may contain reagents made with human serum or plasma. The serum or plasma used has been heat inactivated unless otherwise stated. Handle all sera and kits used as if they contain infectious agents. Observe established precautions against microbiological hazards while performing all procedures and follow the standard procedures for proper disposal of specimens.

CHEMICAL HAZARD:
Material Safety Data Sheets (MSDS) are available for all components of this kit. Review all appropriate MSDS before performing this assay. Avoid all contact between hands and eyes or mucous membranes during testing. If contact does occur, consult the applicable MSDS for appropriate treatment.

7 SPECIMEN COLLECTION AND PREPARATION

- Human serum must be used with this assay. Whole blood or plasma cannot be tested directly.
- Remove serum from the clot of red cells as soon as possible to avoid hemolysis.
- Testing should be performed as soon as possible after collection. Do not leave sera at room temperature for prolonged periods.
- Serum should be used and the usual precautions for venipuncture should be observed. The samples may be stored at 2-8°C for up to 7 days, or frozen at -20°C or lower for up to 30 days. To maintain long-term longevity of the serum, store at -70°C. Avoid repeated freezing and thawing of samples.
- Do not use hemolyzed or lipemic samples.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use.
- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.

8 TEST PROCEDURE

Bring all kit reagents and specimens **to room temperature (~25°C) before use**. Thoroughly mix the reagents and samples before use by gentle inversion.

Note: For long-term storage, all serum, including the experimental, cannot be repeatedly thawed and frozen. Sera should be further aliquoted in a smaller volume and stored at –70°C.

8.1 Preparation of Reagents

1X Wash Buffer

Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water.

To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved.

After diluting to 1X, store at room temperature for a maximum of four months.
Note: Discard the 1X Wash Buffer if you see any microbial growth.

Microtitration Wells

Select the number of coated wells required for the assay. The remaining unused wells should be covered and placed back quickly into the pouch and stored at 2-8°C until ready to use or expiration.

8.2 Assay Procedure

Allow all reagents to reach room temperature (~25°C) and mix thoroughly by gentle inversion before use. Standards, controls and unknown serum to be tested should be assayed in duplicate.

IgG Assay:

1. Mark the microtitration strips to be used.
   Note that the West Nile Antigens (WNRA) and control antigens (NCA) are already bound to the plate in the same arrangement as described in the following table.

West Nile Antigen	Strip #1	Strip #2
A	WNRA	WNRA
B	WNRA	WNRA
C	WNRA	WNRA
D	WNRA	WNRA
E	NCA	NCA
F	NCA	NCA
G	NCA	NCA
H	NCA	NCA

2. In a small, polypropylene tube prepare a 1:300 dilution of the serum sample(s), positive and negative controls in Sample Dilution Buffer for IgG.
3. Add 50 µL of each diluted serum sample to each well. An exemplary arrangement for one serum sample using only one microtiter strip is shown below.
   Note: Samples and controls are to be assayed in WNRA and NCA coated wells.

	Strip #1	Strip #2
	Serum Sample	
A	IgG N	Test Sample #1
B	IgG N	Test Sample #1
C	IgG P	Test Sample #2
D	IgG P	Test Sample #2
E	IgG P	Test Sample #2
F	IgG P	Test Sample #2
G	IgG N	Test Sample #1
H	IgG N	Test Sample #1

4. Cover the strips and incubate for one hour at 37°C in a humidity chamber.
5. Wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer.
6. Add 50 µL of "Ready to Use Enzyme Conjugate-HRP" to each well.
7. Cover the strips and incubate for one hour at 37°C in a humidity chamber.
8. After the incubation, wash the strips six (6) times with the 1X Wash Buffer using an automatic plate washer.
9. Add 150 µL per well of EnWash and incubate for 5 minutes at room temperature (~25°C).
10. After the incubation, wash the strips six (6) times with the 1X Wash Buffer.
11. Add 75 µL of "Liquid TMB Substrate" to each well.
12. Cover the strips and incubate at room temperature (~25°C) in a dark container for 10 minutes.
13. Stop the reaction by adding 50 µL of "Stop Solution" to each well.
14. Read the plate immediately at 450 nm.

9 RESULTS

Results may vary from lot to lot. The results below are given strictly for guidance purposes only.

Applicable for spectrophotometric readings:

Interpretation of Results- IgG Assay

The patient's Immune Status Ratio (ISR) is interpreted as follows:

Compute the average of the two sample replicates with the WNR antigen, the two sample replicates with the NC antigen, and calculate the WNRA/NCA ratio (ISR). Repeat for all samples. Likewise, compute the averages of the positive and negative control replicates with the two antigens and the corresponding ISR.

The ISR for the positive control should be greater than 3.0, while the ISR for the negative control should be less than 1.5.

An ISR of test sample less than 2.0 for the IgG assay should be presumed negative.

An ISR of greater than 3.0 should be presumed positive.

An ISR of between 2.0 and 3.0 should be considered equivocal. For the equivocal result, the assay should be repeated in triplicate.

ISR	Results	Interpretation
≤ 2.0	Negative	No detectable IgG antibody by the ELISA test
2.0 - 3.0	Equivocal	Need confirmatory test
≥3.0	Positive	Indicates presence of detectable IgG antibody. Recommend supplemental confirmatory testing.

False positive results have been reported to occur with certain conditions including, but not limited to, syphilis patients.

Note also that WNRA/NCA ratios (ISR) >2.0 arising from low optical densities (OD) in both the WNRA and NCA wells must be considered potential false positives. See Example #1 in Exclusion Criteria.

Exclusion Criteria:

Calculate the mean Negative Control values with WNRA and with the NCA:

Example: Negative Control (NC) OD

	WNRA	NCA
No 1	0.135	0.126
No 2	0.125	0.110
Total	0.260	0.236

Averages (WNRA) = 0.260 ÷ 2 = 0.130

(NCA) = 0.236 ÷ 2 = 0.118

Calculate the WNRA/NCA ratio: 0.130 ÷ 0.118 = 1.10

Any Negative Control WNRA/NCA ratio greater than 1.50 indicates that the test procedure must be repeated.

Calculate the Positive Control values with WNRA and with the NCA.

Example: Positive Control (PC) OD

	WNRA	NCA
No 1	0.635	0.190
No 2	0.655	0.178
Total	1.290	0.368

Averages (WNRA) = 1.290 ÷ 2 = 0.645

(NCA) = 0.368 ÷ 2 = 0.184

Calculate the WNRA/NCA ratio: 0.645÷ 0.184 = 3.5

Any Positive Control WNRA/NCA ratio less than 3.0 indicates that the test procedure must be repeated.

The results in the table below must be obtained for discrimination capacity of the assay: Non-fulfillment of these criteria is an indication of deterioration of reagents or an error in the test procedure and the assay must be repeated.

Factor	Tolerance
Mean Negative Control Reading (NC)	< 0.400
Mean Positive Control (PC) Reading	> 0.400
PC Immune Status Ratio (ISR)	> 3.000
NC Immune Status Ratio (ISR)	< 1.500

Interpretation of the results:

1. Samples with ISR > 3.0 are considered to be "Positive"
2. Any "Positive" sample must be repeated to verify the result.
3. Samples with ISR <2.0 are considered to be "Negative".
4. Samples with ISR <3.0 but >2.0 are considered to be "Indeterminate" but possible positives, and should be repeated in triplicate or more.
5. ISR >2.0 arising from low optical densities in both the WNRA and NCA wells must be considered potential false positives.

Example #1: Low optical density samples:

Serum Sample	OD	
	WNRA	NCA
No 1	0.044	0.019
No 2	0.016	0.007
Total	0.060	0.026

Averages (WNRA) = 0.060 ÷ 2 = 0.030

(NCA) = 0.026 ÷ 2 = 0.013

Calculate the WNRA/NCA ratio: 0.030 ÷ 0.013 = 2.31

While the ISR is >2.0, this sample must be considered a potential false positive, due to the low optical densities and high relative standard deviations.

This can occur when the plate reader subtracts relatively large values for the "blanks". It is important to not subtract the background from the OD readings.

10 LIMITATIONS

- Samples that generate high optical densities in the Antigen Control (non-WNRA) and thus ISR <3.0 may be false negatives. Diluting the sera further and re-testing may indicate the true ISR.
- Since this is an indirect screening method, the presence of false positive and negative results must be considered.
- All reactive samples must be evaluated by a confirmatory test.
- The reagents supplied in this kit are optimized to measure WNRA reactive antibody levels in serum or plasma.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.

- Hemolyzed and lipemic specimens may give false values and should not be used.

11 PERFORMANCE CHARACTERISTICS

11.1 Sensitivity

In process.

11.2 Specificity

All well confirmed West Nile sera were positive by the West Nile ELISA System. As a control, a number of normal sera and sera infected with unrelated disease were tested. All produced OD450 values which were below the cut-off value.

11.3 Interference

A small percentage of uncharacterized plasma samples containing rheumatoid factor were found to give ISR > 3.0 (West Nile positive) in the IgG assay.

Patients who have St. Louis or Japanese Encephalitis may have a positive result with the West Nile Assay.

12 REFERENCES / LITERATURE

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SYMBOLS USED WITH DRG ASSAYS

Symbol	English	Deutsch	Français	Español	Italiano
	Consult instructions for use	Gebrauchsanweisung beachten	Consulter les instructions d'utilisation	Consulte las instrucciones de uso	Consultare le istruzioni per l'uso
CE	European Conformity	CE-Konfirmitäts-kennzeichnung	Conformité aux normes européennes	Conformidad europea	Conformità europea
IVD	In vitro diagnostic device	In-vitro-Diagnostikum	Usage Diagnostic in vitro	Para uso Diagnóstico in vitro	Per uso Diagnostica in vitro
RUO	For research use only	Nur für Forschungszwecke	Seulement dans le cadre de recherches	Sólo para uso en investigación	Solo a scopo di ricerca
REF	Catalogue number	Katalog-Nr.	Numéro de catalogue	Número de catálogo	Numero di Catalogo
LOT	Lot. No. / Batch code	Chargen-Nr.	Numéro de lot	Número de lote	Numero di lotto
	Contains sufficient for <n> tests/	Ausreichend für "n" Ansätze	Contenu suffisant pour "n" tests	Contenido suficiente para <n> ensayos	Contenuto sufficiente per "n" saggi
	Storage Temperature	Lagerungstemperatur	Température de conservation	Temperatura de conservación	Temperatura di conservazione
	Expiration Date	Mindesthaltbarkeits-datum	Date limite d'utilisation	Fecha de caducidad	Data di scadenza
	Legal Manufacturer	Hersteller	Fabricant	Fabricante	Fabbricante
Distributed by	Distributor	Vertreiber	Distributeur	Distribuidor	Distributore
Content	Content	Inhalt	Conditionnement	Contenido	Contenuto
Volume/No.	Volume / No.	Volumen/Anzahl	Volume/Quantité	Volumen/Número	Volume/Quantità

Symbol	Portugues	Dansk	Svenska	Ελληνικά
	Consulte as instruções de utilização	Se brugsanvisning	Se bruksanvisningen	Εγχειρίδιο χρήστη
CE	Conformidade com as normas europeias	Europaeisk overensstemmelse	Europeisk överensstämmelse	Ευρωπαϊκή Συμμόρφωση
IVD	Diagnóstico in vitro	In vitro diagnostik	Diagnostik in vitro	in vitro διαγνωστικό
RUO				
REF	Catálogo n.º	Katalognummer	Katalog nummer	Αριθμός καταλόγου
LOT	No do lote	Lot nummer	Batch-nummer	Αριθμός Παρτίδος
		Indeholder tilsttrækkeligt til "n" test	Innehåller tillräckligt till "n" tester	Περιεχόμενο επαρκές για «n» εξετάσεις
	Temperatura de conservação	Opbevarings-temperatur	Förvaringstempratur	Θερμοκρασία αποθήκευσης
	Prazo de validade	Udløbsdato	Bäst före datum	Ημερομηνία λήξης
	Fabricante	Producent	Tillverkare	Κατασκευαστής
Content	Conteúdo	Indhold	Innehåll	Περιεχόμενο
Volume/No.	Volume/Número	Volumen/antal	Volym/antal	Όγκος/αριθ..