DRG® Japanese Encephalitis IgG ELISA (EIA-4518)

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REVISED 5 MAY 2011 RM (VERS. 2.1) **RUO IN THE USA**

THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

INTENDED USE

The JE IgG test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA assay system for the detection of antibodies in human serum to JEV derived recombinant antigen (JERA) (1-4).

It is not intended to screen blood or blood components and is for research use only. This kit has not been optimized for vaccine induced seroconversion studies.

PRINCIPLE OF THE TEST

The JE IgG ELISA consists of one enzymatically amplified "two-step" sandwich-type immunoassay.

In this assay, JE IgG Positive Control (Represents reactive or equivocally reactive serum), JE Negative Control (Represents non-reactive serum), and unknown serum samples are incubated in microtitration wells. The serum samples are diluted with Sample Dilution Buffer for IgG. After incubation and 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.

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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 absorbencies of the JERA and the control wells accurately determines whether antibodies to JEV are present.

MATERIALS SUPPLIED

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

JE IgG Assay-specific materials:

1. Coated **Microtiter Strips** for JEV Human IgG:
   Strip holder in ziplock foil, containing 96 polystyrene microtiter wells coated with monoclonal antibody bound to recombinant JERA (Rows A, B, C and D) and Control antigen NCA (Rows E, F, G and H).
   Store at 2-8ºC until ready to use.
2. **Sample Dilution Buffer** for IgG:
   One bottle, 25 mL, for serum sample dilution.
   Store at 2-8ºC until ready to use.

3. JE IgG **Positive Control**:
   One vial, 50 µL.
   The JE IgG Positive Control will aid in monitoring the integrity of the kit as well.
   Store at 2-8°C until ready to use.

4. JE **Negative Control**:
   One vial, 50 µL.
   The JE Negative Control will aid in monitoring the integrity of the kit as well.
   Store at 2-8°C until ready to use.

5. **Ready to Use Enzyme Conjugate-HRP for JE IgG:** One bottle, 6 mL of a pre-diluted goat anti-human IgG-HRP conjugate to be used as is in the procedure below. Store at 2-8°C until ready to use.

   Note: The conjugate should be kept in a light-protected bottle at all times as provided.

6. **10X Wash Buffer**:
   One bottle, 120 mL of Wash Buffer to be used in all the washing steps of this procedure.
   Store at 2-8ºC 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 at 2-8ºC 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.
   Note: The substrate should be kept in a light -protected bottle at all times as provided.

9. **Stop Solution**:
   One bottle, 6 mL to be used to stop the reaction. Store at 2-8ºC until ready to use.
   Caution: Stop Solution contains strong acid, wear protective gloves, mask and safety glasses. Dispose of all materials according to safety rules and regulations.

MATERIALS REQUIRED BUT NOT SUPPLIED

- Microtitration plate reader capable of absorbance measurement at 450 nm
- Biological or High-Grade Water
- Vacuum Pump, Plate Washer
- 37°C Incubator
- 1-10 µL Single-Channel Pipetters, 50-200 µL Single-and Multi-Channel Pipetters.

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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 pipette 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.

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.
- Frozen samples should be thawed to room temperature and mixed thoroughly by gentle swirling or inversion prior to use. Always quick spin before use.

- If sera are to be shipped, they should be packed in compliance with Federal Regulations covering transportation of infectious agents.
- Do not use sera if any indication of growth is observed.

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. Always quick spin serum samples contained in vials or tubes to collect sample at the bottom.

Preparation for Assay

- Preparation of 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 6 months. Use 300 µL/well for each wash cycle.

Note: *Determine if any precipitate, microbial growth or turbidity is found in the 1X Wash Buffer solution before use. Do not use the 1X Wash Buffer if such contamination is found.*

- Coated Microtiter Strips

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

Assay Procedure

Important: Carefully review the table below to understand the JERA and NCA organization. Rows A, B, C, and D are coated with JERA while rows E, F, G and H have been coated with NCA.

Positive, negative, and unknown serum to be tested should be assayed in duplicate. Refer to flow chart at the end of this section for illustration of this procedure.

1. Mark the Coated Microtiter Strips to be used.
2. Dilute your test sera, the JE Negative Control, and the JE IgG Weak Positive Control to 1/300 using the provided Sample diluent.
   Note: You may use small polypropylene tubes for these dilutions and use at least 3 µL of test sera and JE Negative Control and JE IgG Positive Control; for example add 3 µL serum to 897 µL of "Sample Dilution Buffer" for IgG).

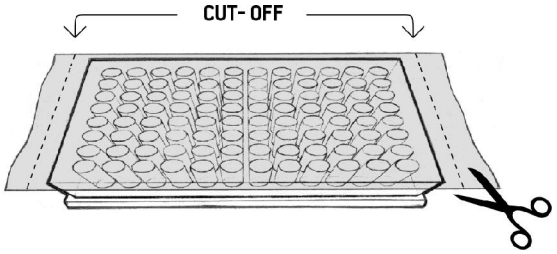
3. Apply the 50 µL/well of 1/300 diluted test sera, JE Negative Control, and JE IgG Weak Positive Control to the plate by multipipettor.

An exemplary arrangement for twenty-two test serum samples in duplicate is shown below.

Example for Serum Sample Application												
	1	2	3	4	5	6	7	8	9	10	11	12
A	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
B	JE Negative Control.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
C	JE IgG Positive Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
D	JE IgG Positive Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
E	JE IgG Positive Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
F	JE IgG Positive Control	S#2	S#4	S#6	S#8	S#10	S#12	S#14	S#16	S#18	S#20	S#22
G	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21
H	JE Negative Control	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21

Note: Rows A-D are precoated with Japanese Encephalitis Recombinant Antigen (JERA). Rows E-H are pre-coated with Normal Cell Antigen (NCA).

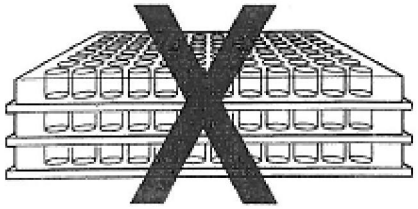
4. Cover the plate with parafilm just on the well opening surface and both sides, so the bottom of the plates is not covered.



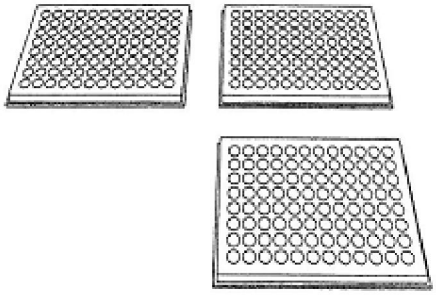
Note: *This is to make sure the temperature distribution is evenly spread out in all wells from bottom and sides; any extra parafilm can be cut-out once the top is sealed to block evaporation.*

5. Incubate the plate at 37°C for 1hour in an incubator.

Note: *Do not stack plates on top of each other. They should be spread out as a single layer. This is very important for even temperature distribution. Do not use any CO2 in the incubator.*



INCORRECT METHOD



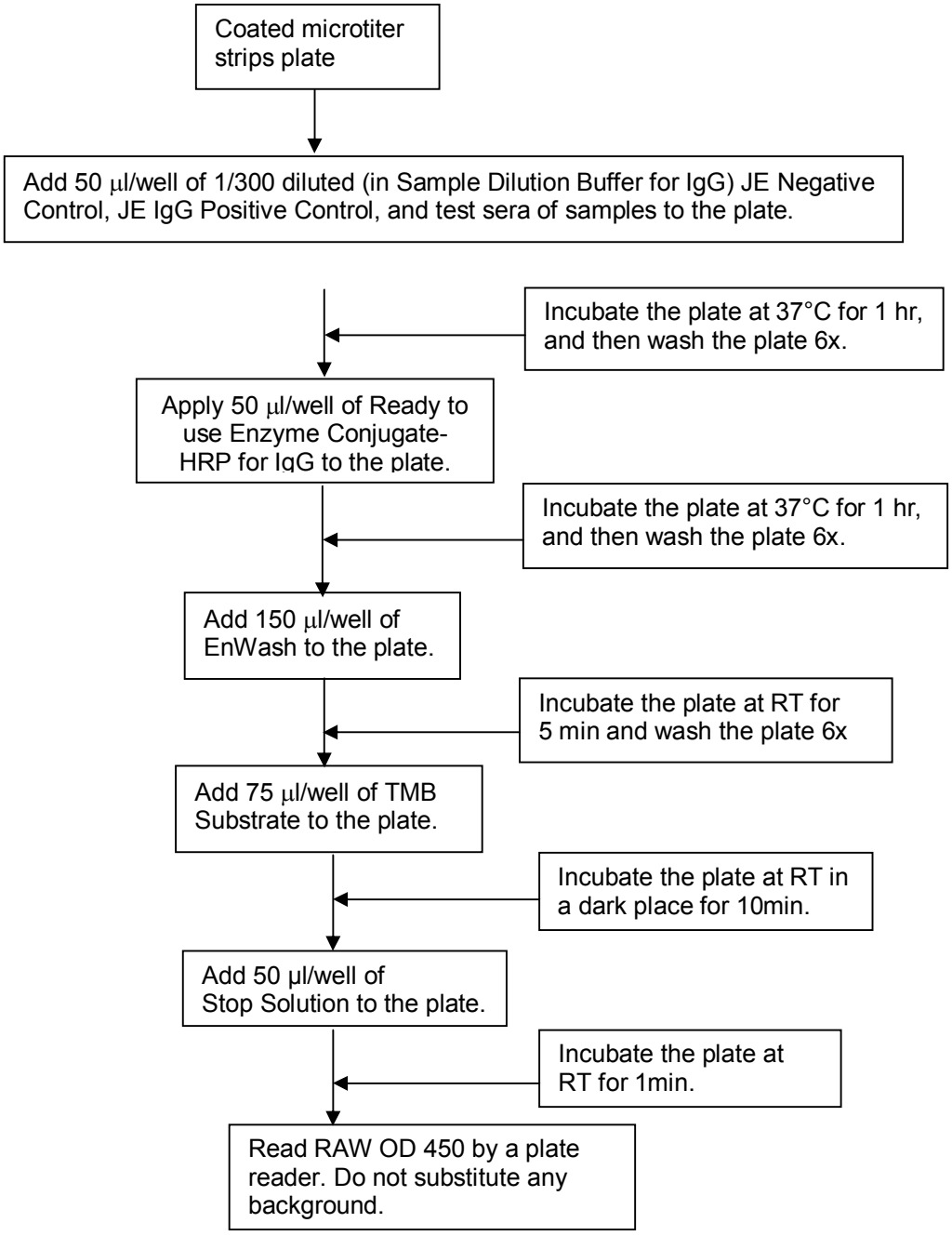
CORRECT METHOD

6. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer (300 µl per well)..
7. Add 50 µl /well of Ready to Use Enzyme-HRP conjugate into all wells by multi-pipettor.
8. Cover the plate with parafilm just on the well opening surface and both sides, so the bottom of the plate should not be covered (see step 4).
9. Incubate the plate at 37°C for 1hour in darkness in an incubator.

Note: *Do not stack plates on top of each other. They should be spread out as a single layer (see step 5). This is very important for even temperature distribution. Do not use* CO_2

10. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer.
11. Add 150 µl /well of EnWash into all wells by multi-pipetter.
12. Incubate the plate at room temperature for 5 minutes without any cover on the plate.
13. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer.
14. Add 75 ml /well of TMB substrate into all wells by multi-pipetter.
15. Place and incubate the plate in a dark place (or container) for 10 minutes without any cover on the plate.
16. After the incubation, add 50 µl /well of Stop solution into all wells by multi-pipetter and incubate at room temperature for 1 minutes without any cover on the plate.
17. After the incubation, read the RAW OD 450 value with a Microplate reader. Please make sure the microplate reader does NOT subtract or normalize any blank values or wells.

JE IgG ELISA for Procedure Flow chart:



QUALITY CONTROL

Each kit contains positive and negative control sera. The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cutoff. The test is invalid and must be repeated if the ISR value of either the controls do not meet the specifications. Acceptable Immune Status Ratio (ISR) values for these controls are found on specification table below. 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. It is recommended that the user refer to NCCLS C24-A and 42 CFR 493.1256 for guidance on appropriate QC practices. The results below are given strictly for guidance purposes only. Applicable for spectrophotometric readings only.

Calculation of the Negative Control:

Calculate the mean JE Negative Control values with JERA and with the Control antigen:

Example: JE Negative Control

	OD	
	JERA	NCA
No 1	0.235	0.230
No 2	0.245	0.224
Total	0.480	0.454

Averages (JERA) = 0.480 ÷ 2 = 0.240

(NCA) = 0.454 ÷ 2 = 0.227

Calculate the JERA/NCA ratio: 0.240 ÷ 0.227= 1.06

Any JE Negative Control JERA/NCA ratio greater than 1.5 indicates that the test procedure must be repeated.

Calculation of the Positive Control:

Calculate JE IgG Positive Control values with JERA and with the NCA.

Example: JE IgG Positive Control

	OD	
	JERA	NCA
No 1	0.938	0.126
No 2	0.898	0.111
Total	1.836	0.237

Averages (JERA) = 1.836 ÷ 2 = 0.918

(NCA) = 0.368 ÷ 2 = 0.119

Calculate the JERA/NCA ratio: 0.918 ÷ 0.119 = 7.71

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

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 JE Negative Control OD in JERA	< 0.400
Mean JE IgG Positive Control OD in JERA	> 0.400
JE IgG Positive Control Immune Status Ratio (ISR)	> 3.000
JE Negative Control Immune Status Ratio (ISR)	< 1.500

CALCULATIONS

(For Unknown Sample Determination):

Calculation of the Immune Status Ratio (ISR): Compute the average of the unknown smaple replciates with the JERA, and the replicates with the NCA, then calute the JERA/NCA ratio (ISR).

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

An ISR of greater than 5.0 for the IgG assay should be presumed positive. The table below summarizes how results should be interpreted.

LIMITATIONS

- For research use only.
- 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 JERA reactive antibody levels in serum.
- Repeated freezing and thawing of reagents supplied in the kit and of specimens must be avoided.
- Serological cross-reactivity across the flavivirus group is common. Certain sera from samples infected with Dengue, West Nile, and Saint Louis, and Rheumatoid sera may give false positive results. Therefore any JE positive sera must be confirmed with other tests.
- This kit has not been optimized for vaccine induced seroconversion studies.

REFERENCES

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