DRG® JE (Japanese Encephalitis) IgM Antibody Capture ELISA (EIA-4505)

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THIS KIT IS INTENDED FOR RESEARCH USE ONLY.

NOT FOR USE IN DIAGNOSTIC PROCEDURES.

1 INTENDED USE

The JE IgM ELISA test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA assay system for measurement of IgM antibodies in human serum to JEV derived recombinant antigen (JERA) (1-4). It is not intended to screen blood or blood components.

2 SUMMARY AND EXPLANATION OF THE TEST

Exposure to JEV causes a disease with a number of symptoms including encephalitis (5-8).
The JE IgM ELISA employs a recombinant antigen called JERA, which can be used as a rapid serological marker for JEV infection. The JERA protein is a recombinant antigen, which consists of a stretch of peptides from different parts of the JE.

3 PRINCIPLE OF THE TEST

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

In this assay, JE Detect Low Control (represents non-reactive serum), JE Detect IgM High Control (represents reactive serum), and unknown serum samples are diluted with Sample Dilution Buffer for JE Detect IgM, then incubated in microtitration wells which have been coated with anti-human IgM antibodies. This is followed by incubation with both JEV derived recombinant antigen (JERA) and Normal Cell Antigen (NCA) separately. After incubation and washing, the wells are treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a third incubation and washing step, the wells are incubated with the tetramethylbenzidine (TMB) substrate.

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.

4 MATERIALS SUPPLIED

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

1. Coated **Microtiter Strips for Human IgM**: Strip holder in foil pouch, containing 96 polystyrene microtiter wells coated with antibody to human IgM in each well.
   Store at 2-8ºC until ready to use.
   The anti-human IgM coated wells are used to capture IgM antibodies from human samples.
2. **Sample Dilution Buffer** for JE IgM Type A:
   One bottle, 25 mL, for serum dilution prior to use in assay
   Store at –2-8º C until ready to use.
   Note: If any precipitate is seen, vortex the tube very well to obtain a homogeneous solution and then use.
3. Ready-to-use **JE Antigen** (JERA) for IgM:
   One tube (3 ml) of a prediluted JERA solution.
   Store at –2-8º C until ready to use.

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4. Ready-to-use **normal cell antigen** (NCA) for IgM:
   One tube (3 ml) of a prediluted NCA solution.
   Store at –2-8º C until ready to use.
5. JE **Low Control**:
   One vial, 50 µL. The JE Detect Low Control will aid in monitoring the integrity of the kit. Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
6. JE IgM **High Control**:
   One vial, 50 µL. The JE Detect IgM High Control will aid in monitoring the integrity of the kit. Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.
7. **10X Wash Buffer**:
   One bottle, 120 mL of Wash Buffer to be used in all the washing steps of this procedure.
   Store at 2-8°C until ready to use.
8. **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 2-8º C until ready to use
9. Ready to Use **Enzyme Conjugate-HRP** for IgM:.
   One bottle, 6 mL of a pre-diluted HRP conjugated flavivirus reactive monoclonal antibody (mAb). 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.
10. Liquid **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.
11. **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.

5 MATERIALS REQUIRED BUT NOT SUPPLIED

- ELISA Spectrophotometer 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 Pipettors.
- Polypropylene tubes
- Parafilm
- Timer
- Vortex

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6 PRECAUTIONS

- All human source materials used in the preparation of controls have tested low for antibodies to HIV 1&2, Hepatitis C and Hepatitis B surface antigen. However, no test method can ensure 100% efficiency. Therefore, all human controls and antigen should be handled as potentially infectious material. The Center for Disease Control and the National Institute of Health recommend that potentially infectious agents be handled at the Biosafety Level 2.
- 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 Ready to Use Enzyme Conjugate HRP for IgM. 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.
- 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.
  Note: *CSF can be used. However, our kit has not been tested or optimized with CSF. Before using the DRG kit, one has to optimize the CSF system.*
- 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.

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- 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: All serum, including the experimental, cannot be repeatedly thawed and frozen.
For long-term storage, sera should be further aliquoted in a smaller volume and stored at -70°C.
Always quick spin serum sample contained in vials or tubes to collect sample at the bottom.

7.1 Preparation of Reagents:

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 up to 4 months. Check for contamination prior to use.

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.

7.2 Assay Procedure:

1. High and low controls should be assayed in duplicate for both JERA and NCA portions of assay. Unknown serum samples to be tested can be assayed singly or in duplicate but must be assayed for both JERA and NCA portions of assay. Up to forty-four test specimens can be tested on one 96-well plate.
2. Mark the microtitration strips to be used.
3. Dilute test sera, and controls to 1/100 using the provided Sample Dilution Buffer.
   Use small polypropylene tubes for these dilutions and at least 4 µL of sera and high and low controls.
   For example: 4 µL serum plus 396 µL of Sample Dilution Buffer for JE IgM to make 1/100 dilution.
4. Apply the 50 µL/well of 1/100 diluted test sera, JE Low Control, and JE IgM High Control to the plate by single or multi-pipettor as appropriate. An exemplary arrangement for twenty-two test serum samples in duplicate is shown below.

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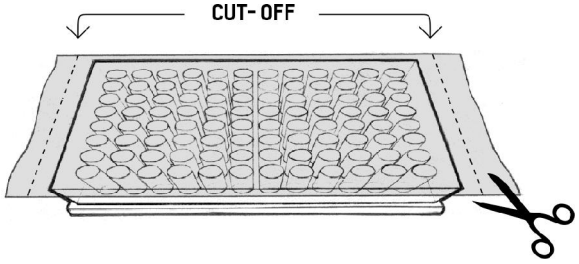
Example for Serum Sample Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	JE Low 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 Low 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 IgM High 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 IgM High 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 IgM High 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 IgM High 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 Low 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 Low Control.	S#1	S#3	S#5	S#7	S#9	S#11	S#13	S#15	S#17	S#19	S#21

5. Cover the plate with parafilm just on the well opening surface, 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.*



6. 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 CO_2 or other gases. Do not place plates in contact with any wet substances such as wet paper towels etc.*
7. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer. Use 300 µl per well in each wash cycle.
8. Add 50µl /well of JERA into row A-D and 50µl /well of NCA into row E-H by multichannel-pipettor.

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An exemplary application for JERA and NCA is shown below.

Example for JE Antigens Application

	1	2	3	4	5	6	7	8	9	10	11	12
A	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
B	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
C	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
D	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA	JERA
E	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
F	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
G	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA
H	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA	NCA

9. Cover the plate with parafilm just on the well opening surface, so the bottom of the plate should not be covered (see step 5).
10. Incubate the plate at 37°C for 1 hour in an incubator..
11. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer. Use 300 µl per well in each wash cycle.
12. Add 50µl /well of ready to use Enzyme-HRP conjugate into all wells by multichannel-pipettor.
13. Cover the plate with parafilm just on the well opening surface, so the bottom of the plate should not be covered (see step 5).
14. Incubate the plate at 37°C for 1hour in an incubator in darkness.
15. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer.
16. Add 150µl /well of EnWash into all wells by multichannel-pipettor.
17. Incubate the plate at room temperature for 5 minutes without any cover on the plate.
18. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer.
19. Add 75µl /well of TMB substrate into all wells by multichannel-pipettor.
20. Place and incubate the plate at room temperature in a dark place (or container) for 10 minutes without any cover on the plate.
21. After the incubation, add 50µl /well of Stop solution into all wells by multichannel-pipettor and incubate at room temperature for 1 minute without any cover on the plate.
22. 22. 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.

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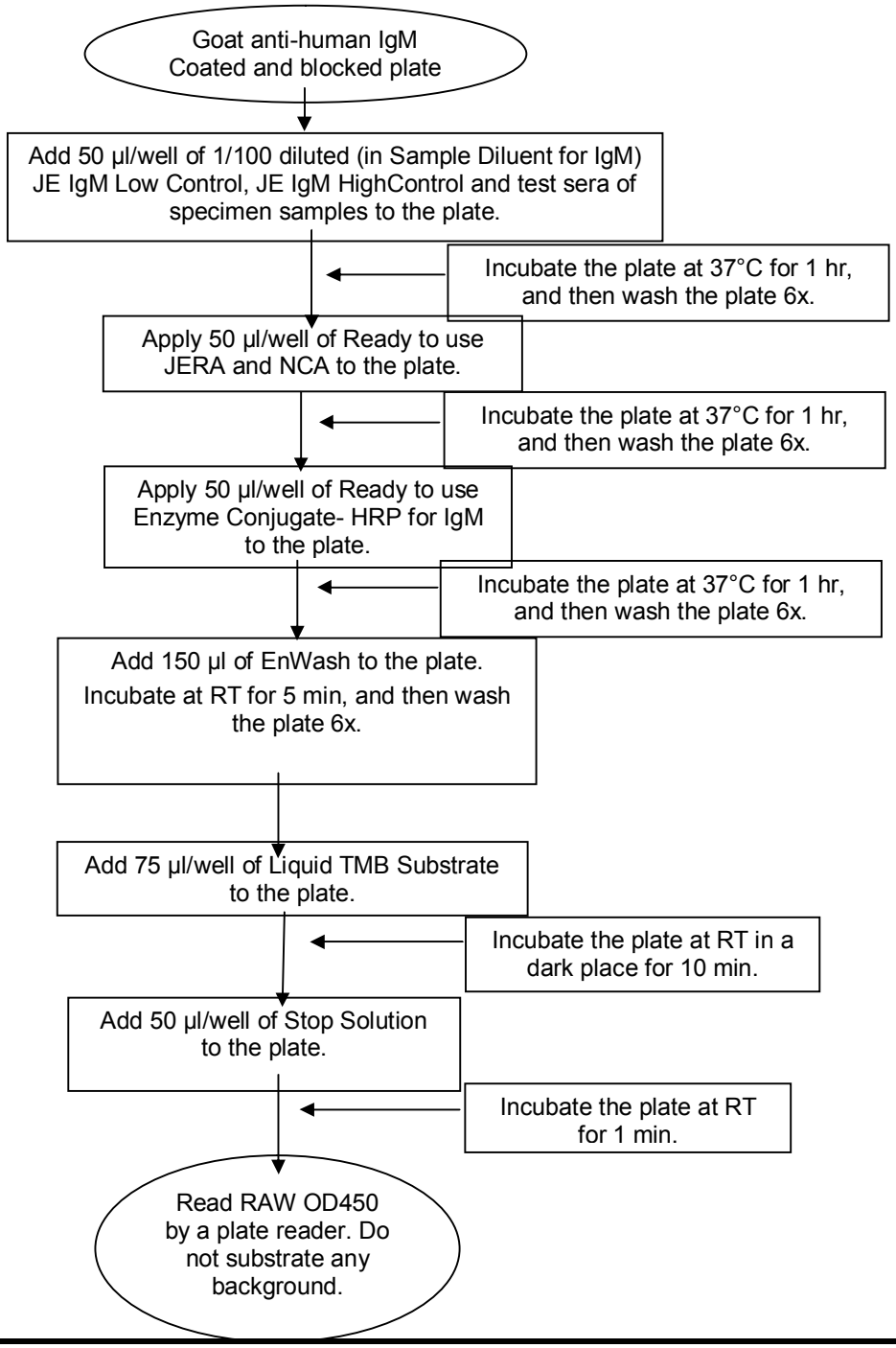
JE IgM Capture ELISA Flow Chart



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8 CALCULATION OF THE LOW CONTROL:

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

Example: JE Low Control

	OD	
	JERA	NCA
No 1	0.188	0.066
No 2	0.192	0.061
Total	0.380	0.254

Averages (JERA) = 0.380 ÷ 2 = 0.190

(NCA) = 0.254 ÷ 2 = 0.127

Calculate the JERA/NCA ratio: 0.190 ÷ 0.127 = 1.50

Any JE Low Control JERA/NCA ratio greater than 2.8 indicates that the test procedure must be repeated.

Calculation of the High Control:

Calculate JE IgM High Control values with JERA and with the NCA.

Example: JE IgM High Control

	OD	
	JERA	NCA
No 1	1.035	0.105
No 2	1.055	0.115
Total	2.090	0.220

Averages (JERA) = 2.090 ÷ 2 = 1.045

(NCA) = 0.220 ÷ 2 = 0.110

Calculate the JERA/NCA ratio: 1.045÷ 0.110 = 9.5

Any JE IgM High Control JERA/NCA ratio less than 6.0 indicates that the test procedure must be repeated.

9 CALCULATIONS

Calculation of the Immune Status Ratio (ISR):

Compute the average of the duplicates of each unknown sample with the JERA, and the average of the duplicates of each unknown sample with the NCA. Then for each individual sample, calculate the ratio of the JERA duplicates average to the NCA duplicates average (JERA/NCA ISR).

10 LIMITATIONS

- The reagents supplied in this kit are optimized to measure JERA reactive antibody levels in serum.
- Serological cross-reactivity across the flavivirus group is common. Certain sera from specimens from those donors infected with Dengue, West Nile, and Saint Louis virus may give false high results. Therefore any JE high sera must be confirmed with other tests.
- In areas where JE and dengue are co-existent, JE high samples should also be assayed for dengue reactivity. Samples with borderline JE positivity and medium to high dengue reactivity could be suspected for dengue infection and require further confirmatory assays.

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- The assay performance characteristics have not been established for visual result determination.
- This kit has not been optimized for vaccine induced seroconversion studies.
- The use of JE Detect IgM kit for vaccine induced seroconversion studies may lead to many "Equivocal" results.

11 REFERENCES

1. Martin, D.A., Muth, D.A., Brown, T., Johnson, A.J., Karabatsos,R, Roehrig, J.T. 2000. Standardization of Immunoglobulin M Capture Enzyme-Linked Immunosorbent Assays for Routine Diagnosis of Arboviral Infections. J. Clin. Microbiol. 38(5):1823-1826.
2. Cardosa MJ, Wang SM, Sum MS, Tio PH. Antibodies against prM protein distinguish between previous infection with dengue and Japanese encephalitis viruses. BMC Microbiol. 2002 May 5;2(1):9
3. Pandey B, Yamamoto A, Morita K, Kurosawa Y, Rai S, Adhikari S, Kandel P, Kurane I. Serodiagnosis of Japanese encephalitis among Nepalese patients by the particle agglutination assay. Epidemiol Infect. 2003 Oct;131(2):881-5.
4. Thakare JP, Gore MM, Risbud AR, Banerjee K, Ghosh SN. ion of virus specific IgG subclasses in Japanese encephalitis patients. Indian J Med Res. 1991 Sep;93:271-6.
5. Lowry PW, Truong DH, Hinh LD, Ladinsky JL, Karabatsos N, Cropp CB, Martin D, and Gubler DJ. Japanese encephalitis among hospitalized pediactric and adult patients with acute encephalitis syndrome in Hanoi, Vietnam 1995. Am. J. Trop. Med. Hyg, 1998;58(3):324-329.
6. Tsai TF. Factors in the changing epidemiology of Japanese encephalitis and West Nile fever. In: Saluzzo JF ed., Factors in the Emergence of Arboviral Diseases. Amsterdam: Elsevier, 1997;179-189.
7. Tsai TF. Japanese encephalitis. In: Feigin RD and Cherry JD (eds.), Textbook of Pediatric Infectious Diseases, 4th edition, Philadelphia: W.B. Saunders, 1997;1993-2001.
8. Rosen L. The natural history of Japanese encephalitis. Annu. Rev. Microbiol., 1986;40:395-414.

Rev. 8/9/12cc