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

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RUO

This kit is intended for Research Use Only.

Not for use in diagnostic procedures.

INTENDED USE

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

It is not intended to screen blood or blood components. This kit has not been optimized for vaccine induced seroconversion studies. For Research Use Only. Not for use in diagnostics.

PRINCIPLE OF THE TEST

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

In this assay, JE Negative Control (Represents non-reactive serum), JE IgM Positive Control (Represents reactive serum), and unknown serum samples are incubated in microtitration wells which have been coated with anti-human IgM antibodies, followed by incubation with both JEV derived recombinant JERA and Normal Cell Antigen (NCA) separately.

The serum samples may be diluted with Sample Dilution Buffer for JE IgM. After incubation and washing, the wells are treated with a JERA-specific antibody labeled with the enzyme horseradish peroxidase (HRP). After a second 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.

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:

JE IgM ELISA-specific materials:

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 Type A IgM:
   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. **JE Negative Control**:
   One vial, 50 µL of heat–inactivated serum.
   The JE Negative Control will aid in monitoring the integrity of the kit as well.
   Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.

4. **JE IgM Positive Control**:
    One vial, 50 µL of heat–inactivated serum.
   The JE IgM Weak Positive Control will aid in monitoring the integrity of the kit as well.
   Store at 2-8°C until ready to use. Quick spin the vial briefly before use to collect the content at the bottom.

DRG International Inc., USA

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5. Ready-to-use **JE Antigen** (JERA) for IgM:
    One tube (3 ml) of Ready-to-use JERA solution.
    Store at 2-8º C until ready to use.

6. Ready-to-use **normal cell antigen** (NCA) for IgM:
    One tube (3 ml) of Ready-to-use NCA solution.
    Store at 2-8º C until ready to use.

7. Ready to Use **Enzyme Conjugate-HRP** for IgM:
    One bottle, 6 mL of a pre-diluted HRP conjugated flavivirus reactive monoclonal antibody (mAb) to be used as is 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.

8. **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.

9. **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.

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.

MATERIALS REQUIRED BUT NOT SUPPLIED

– ELISA Spectrophotometer capable of absorbance measurement at 450 nm
– Biological or High-Grade Water
– Vacuum Pump
– Plate Washer
– Humidified Incubator or Water Bath
– 1-10 µL Single-Channel Pipetters, 50-200 µL Single-and Multi-Channel Pipetters.
– Polypropylene tubes
– Parafilm
– Timer
– Vortex

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PRECAUTIONS

- All human source materials used in the preparation of controls have tested negative 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.

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.

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

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- 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.

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.

Assay Procedure:

1. 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. Twenty-two test specimens can be tested in duplicate 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 diluent.
    Use small polypropylene tubes for these dilutions and at least 4 µL of sera and positive and negative 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 Negative Control, and JE IgM Weak Positive Control to the plate by single or multi-pipetter 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 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 IgM Weak 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 IgM Weak 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 IgM Weak 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 IgM Weak 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

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.
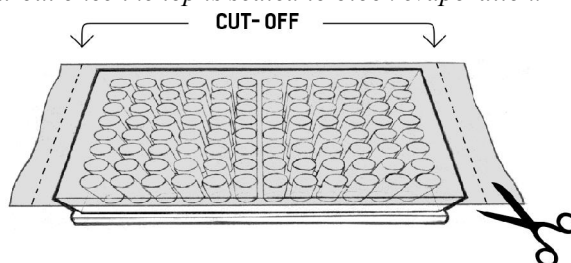
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6. Incubate the plate at 37°C for 1hour in a humidified incubator with water container. Humidification can be achieved using a water tray at the bottom of incubator.
   Note: <u>*Do not stack plates on top of each other.*</u> *They should be spread 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.*
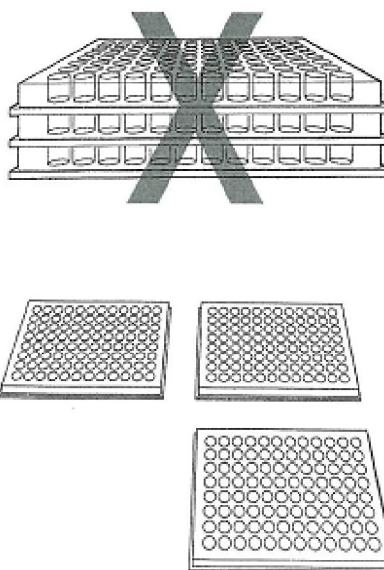
CORRECT METHOD

7. After the incubation, wash the plate 6 times with automatic plate washer using 1x Wash buffer. Use 300 ml 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 multi-pipetter.

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.

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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 multi-pipetter.

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 1 hour 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 multi-pipetter.

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 multi-pipetter.

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 multi-pipetter and incubate at room temperature for 1 minute without any cover on the plate.

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.

CSF application: CSF should be run using undiluted (1:1) samples. If there is not enough volume to test, the CSF samples may be diluted out 1:2 using the Sample Dilution Buffer for JE IgM provided. However, one needs to optimize the proper dilution factor. The rest of the process is the same as described for serum.
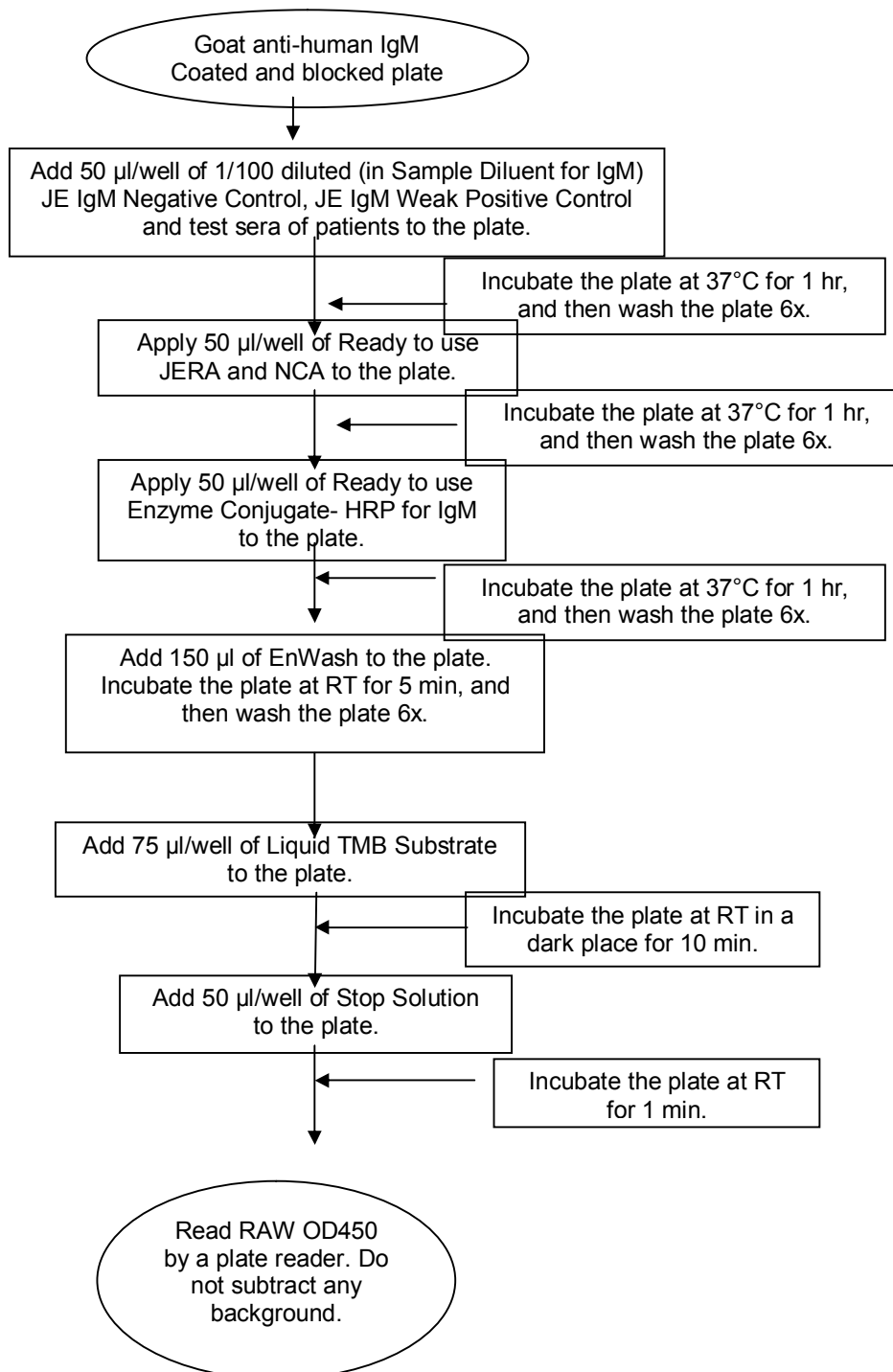
Note: It is necessary to validate the CSF system in a laboratory before using unknown samples.

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JE IgM Capture ELISA Flow Chart

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              ┌─────────────────────────────┐
              │   Goat anti-human IgM        │
              │   Coated and blocked plate   │
              └─────────────────────────────┘
                          │
                          ▼
    ┌──────────────────────────────────────────────────┐
    │ Add 50 µl/well of 1/100 diluted (in Sample        │
    │ Diluent for IgM) JE IgM Negative Control, JE IgM   │
    │ Weak Positive Control and test sera of patients    │
    │ to the plate.                                      │
    └──────────────────────────────────────────────────┘
                          │         ┌──────────────────────────────┐
                          │◄────────│ Incubate the plate at 37°C    │
                          │         │ for 1 hr, and then wash the   │
                          │         │ plate 6x.                     │
                          ▼         └──────────────────────────────┘
    ┌──────────────────────────────────────────────────┐
    │ Apply 50 µl/well of Ready to use                   │
    │ JERA and NCA to the plate.                         │
    └──────────────────────────────────────────────────┘
                          │         ┌──────────────────────────────┐
                          │◄────────│ Incubate the plate at 37°C    │
                          │         │ for 1 hr, and then wash the   │
                          │         │ plate 6x.                     │
                          ▼         └──────────────────────────────┘
    ┌──────────────────────────────────────────────────┐
    │ Apply 50 µl/well of Ready to use                   │
    │ Enzyme Conjugate- HRP for IgM                      │
    │ to the plate.                                      │
    └──────────────────────────────────────────────────┘
                          │         ┌──────────────────────────────┐
                          │◄────────│ Incubate the plate at 37°C    │
                          │         │ for 1 hr, and then wash the   │
                          │         │ plate 6x.                     │
                          ▼         └──────────────────────────────┘
    ┌──────────────────────────────────────────────────┐
    │ Add 150 µl of EnWash to the plate.                 │
    │ Incubate the plate at RT for 5 min, and            │
    │ then wash the plate 6x.                            │
    └──────────────────────────────────────────────────┘
                          │
                          ▼
    ┌──────────────────────────────────────────────────┐
    │ Add 75 µl/well of Liquid TMB Substrate             │
    │ to the plate.                                      │
    └──────────────────────────────────────────────────┘
                          │         ┌──────────────────────────────┐
                          │◄────────│ Incubate the plate at RT in a │
                          │         │ dark place for 10 min.        │
                          ▼         └──────────────────────────────┘
    ┌──────────────────────────────────────────────────┐
    │ Add 50 µl/well of Stop Solution                    │
    │ to the plate.                                      │
    └──────────────────────────────────────────────────┘
                          │         ┌──────────────────────────────┐
                          │◄────────│ Incubate the plate at RT      │
                          │         │ for 1 min.                    │
                          ▼         └──────────────────────────────┘
              ┌─────────────────────────────┐
              │   Read RAW OD450             │
              │   by a plate reader. Do      │
              │   not subtract any           │
              │   background.                │
              └─────────────────────────────┘
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QUALITY CONTROL

Each kit contains positive and negative control sera to ensure assay performance..

The negative and positive controls are intended to monitor for substantial reagent failure. The positive control will not ensure precision at the assay cut-off.

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	0.188	0.129
No 2	0.192	0.125
Total	0.380	0.254

Averages (JERA) $= 0.380 \div 2 = 0.190$

      (NCA) $= 0.254 \div 2 = 0.127$

Calculate the JERA/NCA ratio: $0.190 \div 0.127 = 1.50$

Any JE Negative Control JERA/NCA <u>ratio greater than 2.8</u> indicates that the test procedure <u>must be repeated</u>.

Calculation of the Weak Positive Control:

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

Example: JE IgM Positive Control

	OD	
	JERA	NCA
No 1	0.635	0.105
No 2	0.655	0.115
Total	1.290	0.220

Averages (JERA) $= 1.290 \div 2 = 0.645$

      (NCA) $= 0.220 \div 2 = 0.110$

Calculate the JERA/NCA ratio: $0.645 \div 0.110 = 5.86$

Any JE IgM Weak Positive Control JERA/NCA <u>ratio less than 5.0</u> indicates that the test procedure <u>must be repeated</u>.

The results in the table below must be obtained in order that the results of the run may be reported.
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.

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Factor	Tolerance
Mean JE Negative Control OD in JERA	< 0.300
Mean JE IgM Weak Positive Control OD in JERA	> 0.350
JE IgM Weak Positive Control Immune Status Ratio (ISR)	> 5.000
JE Negative Control Immune Status Ratio (ISR)	< 2.800

CALCULATIONS

Calculation of the Immune Status Ratio (ISR):

Compute the average of the two unknown sample replicates with the JERA, and replicates with the NCA, then calculate the JERA/NCA ratio (ISR). The ISR for the positive control should be greater than 6.0, while the ISR for the negative control should be less than or equivalent to 4.0.

Selection of the Cut-off:

The cut-off was selected using values from a small set of field data and is an estimate only.

Interpretation of Results:

The table below shows how the results should be interpreted.

ISR	Results	Interpretation
< 4.0	Negative	No detectable IgM antibody by the ELISA test
4-6	Equivocal	Need confirmatory test
> 6.0	Positive	Indicates presence of detectable IgM antibody. Recommend supplemental confirmatory testing.

LIMITATIONS

- For research use only. Not for use in diagnostic procedures.
- 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
- Serological cross-reactivity across the flavivirus group is common. Certain sera from samples infected with Dengue, West Nile, and Saint Louis virus may give false positive results. Therefore any JE positive sera must be confirmed with other tests.
- In areas where JE and dengue are co-existent, JE positive 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.
- The assay performance characteristics have not been established for visual result determination.
- Results from immunosuppressed patients must be interpreted with caution.
- This kit has not be optimized for vaccine induced seroconversion studies.
- The use of this JE IgM kit for vaccine induced seroconversion studies may lead to many "Equivocal" results.

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