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JE IgM ANTIBODY CAPTURE ELISA
ELISA

REF

Catalog No:E6-151

For Research Use Only

INTENDED USE

The Immunospec JE ELISA test for exposure to Japanese Encephalitis Virus (JEV) is an ELISA assay system for the ion of IgM antibodies in human serum to JEV derived recombinant antigen (JERA) (1-4). This test is to aid in the diagnosis of human exposure to the Japanese Encephalitis Virus (JEV). It is not intended to screen blood or blood components, and is for research use only.

SUMMARY AND EXPLANATION OF TEST

Exposure to JEV causes a disease with a number of symptoms including encephalitis (5-8). The *JE* 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 JEV antigens.

PRINCIPLE OF THE TEST

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

In this assay, JE Negative Control (Represents non-reactive serum), JE IgM Weak 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 ELISA Kit contains sufficient reagents for one plate of 96 wells (12 x 8 strips) each. The kit contains the following reagents:

JE ELISA-specific materials:

1. **Anti-Human IgM Coated Microtiter Strips:** Strip holder with cover, containing 96 polystyrene microtiter wells coated with antibody to human IgM in each well. Store at 2-8ºC until ready to use.
2. **Sample Dilution Buffer for JE IgM:** One bottle, 25 mL, for ELISA plate wells in IgM 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 Ready-to-use JERA solution. Store at –2-8º C until ready to use.
4. **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.
5. **JE Negative Control:** One vial, 30 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 for up to 7 days. 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 –70°C.
6. **JE IgM Weak Positive Control:** One vial, 30 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 for up to 7 days. 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 –70°C.
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. **Ready to Use Enzyme Conjugate-HRP for IgM:** One bottle, 6 mL of a pre-diluted conjugate to be used as is in the procedure below. Store at 2-8°C until ready to use.
9. ***En*Wash:** One bottle, 20 mL of *En*Wash 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

PRECUTIONS

- 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 Immunospec's 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.
- 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 sample contained in vials or tubes to collect sample at the bottom.

Preparation of Reagents:

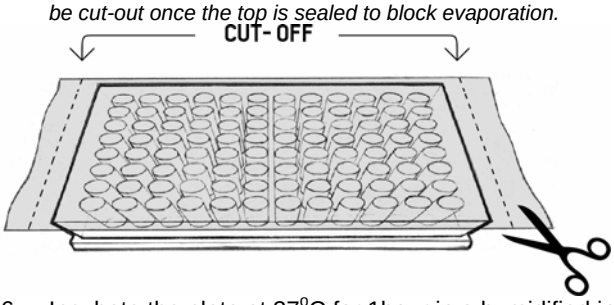
- Preparation of 1X Wash Buffer
  Dilute the 10X Wash Buffer to 1X using Biological or High-Grade Water. To prepare a 1X wash buffer solution, mix 120 ml 10X wash buffer with 1080 ml distilled (or deionized) water and rinse out any crystals. Swirl until well mixed and all crystals are dissolved. After 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:

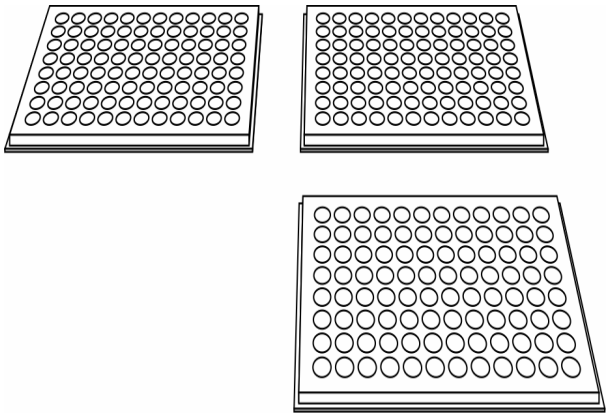
1. Positive, negative and unknown serum to be tested should be assayed in duplicate. Refer to flow chart at the end of this section for illustration of this procedure. Twenty-two test specimens can be tested in duplicate on one 96 well plate.
2. Mark the microtitration strips to be used.
3. Dilute test 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.

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.*



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**: 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.*



CORRECT METHOD

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 multi-pipetter.

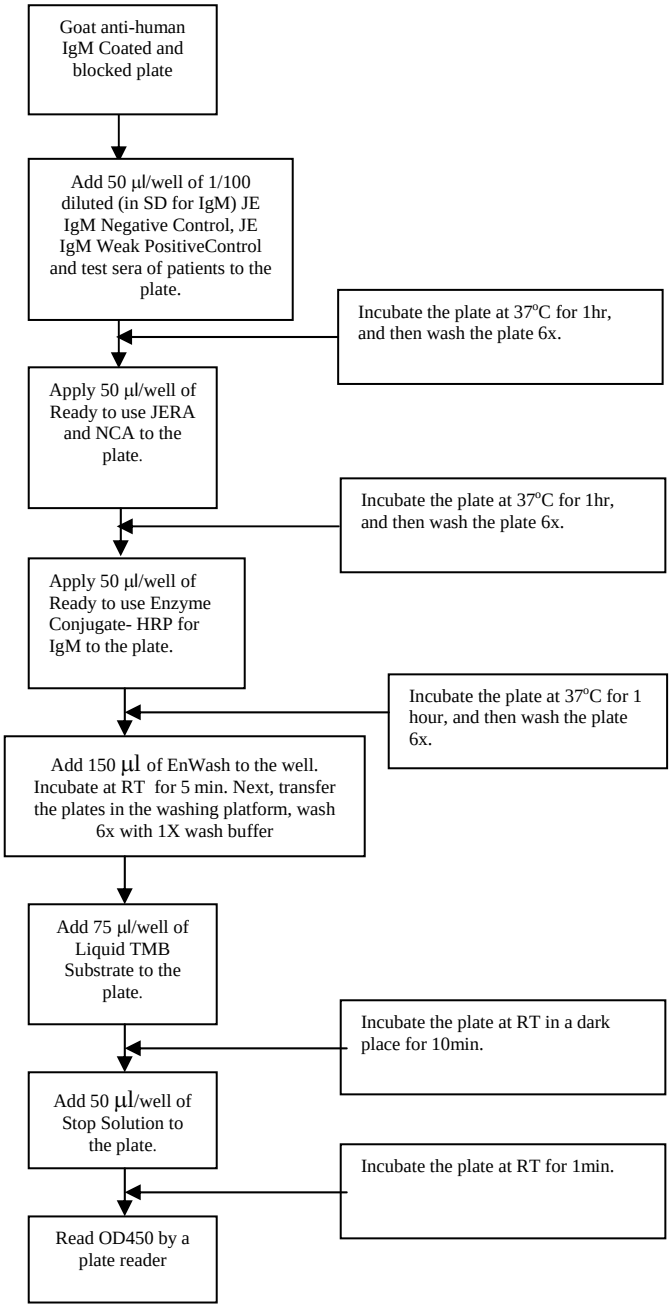
An exemplary application for JERA and NCA is shown below.

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 a humidified incubator with a water container for humidification (see step 6).
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 1hour in a humidified incubator (see step 6).
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 Liquid 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 OD 450 value with a Microplate reader.

JE ELISA Procedure Flow chart:



QUALITY CONTROL

Each kit contains positive and negative control sera. Acceptable Immune Status Ratio (ISR) values for these controls are found on specification table below. 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. If the test is invalid, patient results cannot be reported. 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	0.108	0.066
No 2	0.082	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 Negative Control JERA/NCA ratio greater than 2.8 indicates that the test procedure must be repeated.

Calculation of the Weak Positive Control: Calculate JE IgM Weak Positive Control values with JERA and with the NCA.

Example: JE IgM Weak 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 ÷ 2 = 0.645
(NCA) = 0.220 ÷ 2 = 0.110

Calculate the JERA/NCA ratio: 0.645÷ 0.110 = 5.86

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

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.

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 negative control replicates with the JERA, the two negative control replicates with the NCA, and calculate the JERA/NCA ratio (ISR). Likewise, compute the averages of the two weak positive control replicates and the two sample replicates with the two antigens and the corresponding ISR. The ISR for the weak 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
6.0	Negative	No able IgM antibody by the ELISA test
6-10	Equivocal	Need confirmatory test
>10	Positive	Indicates presence of able IgM antibody. Recommend supplemental confirmatory testing.

LIMITATIONS

- 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 patients 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.
- The assay performance characteristics have not been established for visual result determination.
- Results from immunosuppressed patients must be interpreted with caution.
- Assay results should be interpreted only in the context of other laboratory findings and the total clinical status of the patient.

PERFORMANCE CHARACTERITICS
Sensitivity and Specificity Studies:

The following specificity and sensitivity of the JE ELISA kit was derived from two small studies. One was a comparison conducted by the CDC, while the second a JE infected human serum panel provided by CDC. An OD ratio of each sample was calculated (OD450 on JERA/OD450 of control at each dilution). A ratio >6 is considered positive for JE infection.

		Positive	*Convalescent*	*Negative*	*Total*
JE ELISA	+	31	0	0	31
	-	0	1	196	197
Total		31	1	196	228

Note: Specificity panel includes normal, and other disease sera, such as sera from patients with autoimmune diseases (ANA, RF, etc; not including Dengue, and WNV, and SLE sera). Limited studies with dengue sera showed cross-reactivity with some sera. One convalescent serum did not show any IgM reactivity.

Serological Sensitivity: 31/31, or 100%
Serological Specificity: 0/196, or 100%

Cross-reactivity Studies:

The table below shows the results for the cross-reactivity study performed with the JE ELISA kit.

Tested positive serum	***Total specimens***	***Positive***	***Positive and Equivocal result***
Normal (North American)	110	0	0/110
Rheumatoid Factor	8	0	0/8
Anti-nuclear Antibody	10	0	0/10
Cytomegalovirus	10	0	0/10
Epstein-Barr virus	15	0	0/15
Varicella-zoester virus	10	0	0/10
Hepatitis B virus	9	0	0/9
Hepatitis C virus	19	0	0/19
Malaria	5	0	0/5

Interference Study:
Eight plasma samples containing high levels (860 -5630 IU) of Rheumatoid factor gave negative results in the IgM assay.

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