Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Standard Operating Procedure (SOP) No.	JE 008
Prepared Date:	9 Jan 2010
Effective Date:	11 Jan 2010
Total No. of copies:	2
Distribution:	National Measles Laboratory, NPHL-1 copy BP Koirala Institute of Health Sciences-1 copy
Review Period:	2 years

APPROVED BY

Title	Signature and Name	Date
Document Author	…….. Supriya Sharma Microbiologist, NPHL	9 Jan 2010
Authorized by	…….. Dr Geeta Shakya Director, NPHL	11 Jan 2010

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: JE IgM ELISA using AFRIMS kits for CSF and serum samples

SOP CODE.: JE 008

1. OBJECTIVE: This standard operating procedure provides guideline for detection of IgM antibodies to Japanese Encephalitis in serum and CSF collected from Acute Encephalitis Syndrome (AES) patients.

2. SCOPE: Detection of IgM antibodies to Japanese Encephalitis in serum and CSF collected from Acute Encephalitis Syndrome (AES) patients can be done using AFRIMS kit.

3. INTRODUCTION:

The AFRIMS ELISA kit is used for the detection of IgM antibodies to Japanese encephalitis in serum and CSF in patients with acute encephalitic syndrome (AES) cases.

Japanese encephalitis (JE) are mosquito borne viruses belonging to the family Flaviviridae and the serological complex of Japanese encephalitis. JE is a common cause of human viral encephalitis in temperate and tropical regions throughout the world. JE has been implicated in periodic outbreaks of encephalitis cases throughout Asia and the Western Pacific countries and hence poses a major public health problem. JE infection has an incubation period of 6-12 days and results in an acute illness with symptoms including fever, convulsions and a depressed level of consciousness and coma. With JE infection there is a high fatality rate and high prevalence of neurological sequelae in those who survive the acute illness. Though JE infections can have serious side effects there is a high ratio of asymptomatic to symptomatic infections. JE is transmitted to humans by infected mosquitoes and is maintained in infected invertebrate reservoirs. In domestic animals such as swine and horses, JE infection causes still births, encephalitis and occasionally death.

4. REQUIREMENTS:

4.1 Reagents required formulating 1000 JE EIA Kits

Reagent Class	Reagent Type	Storage Temp	No. of Vials	Vial Volume
Plate coat	Goat anti-human IgM	2ºC to 8ºC	1	1.0 ml
Virus antigen	JE virus Hemagglutinin (Sucrose-acetone extracted non-infectious)	-85ºC to -60ºC	2	1.0 ml
Normal Human Serum (Acetone extracted)	13.5%NHS (acetone extracted) in PBS	-25ºC to -15ºC	6	40 ml
Serum standard (Positive control)	Weak anti-JEV IgM	-25ºC to -15ºC	1	0.3 ml
Normal control	Normal Human Serum (NHS)	-25°C to -15°C	1	0.3 ml
Conjugate	Human anti-flavivirus IgG-HRP	-25°C to -15°C	1	0.6 ml
Chromogen	o-phenylenediamine (OPD)	2°C to 8°C	1	0.5 gm
Blocking reagent	Bovine serum albumin, FxV	2°C to 8°C	1	2.0 gm

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Equipment	Consumables	Registers
Micropipettes 5-1000ul Multichannel pipette 50-100ul Incubator ELISA plate washer ELISA reader Electric Weighing Balance pH Meter Magnetic Stirrer	Distilled water Tissue Paper Yellow Box, Ice packs Micropipette tips Gloves, Marker Autoclavable Discarding Bin, 1% Hypochlorite solution	Japanese Encephalitis register Requisition File Acknowledgement slip

5. RESPONSIBILITIES:

It is the responsibility of the laboratory personnel to properly test the specimens and report the result accordingly to NPHL, Teku and WHO-IPD, Chakupat.

6. DESIGNATED AREA: National Measles/JE Laboratory, Teku, Kathmandu, Nepal

7. PROCEDURE:

DAY I

7.1. Sensitization Step:

7.1.1.Prepare **0.018 M Carbonate buffer** pH 9.0±0.1

Sodium Carbonate (Na_2CO_3)	0.076	gm
Sodium Bicarbonate ($NaHCO_3$)	0.700	gm
MQ water to	500	ml

7.1.2.Prepare **1X PBS** pH 7.4±0.1

Prepare 10X PBS

Sodium Chloride (NaCl)	160.0	gm
Potassium Chloride (KCl)	4.0	gm
Potassium Phosphate, monobasic, anhydrous (KH_2PO_4)	3.8	gm
Sodium Phosphate, dibasic, anhydrous (Na_2HPO_4)	18.0	gm
Final volume of distilled water to	2000	ml

Prepare 1X PBS

10X PBS	200	ml
Distilled water	1800	ml
Check pH 7.4±0.1, adjust pH by adding 1N NaOH		

Prepare wash buffer solution (PBST)

10X PBS	50	ml
Distilled water	950	ml
Tween 20	500	ul

7.1.3. Sensitization:

- Bring the Carbonate buffer pH 9.0±0.1 and reconstituted goat anti-human IgM at room temperature (15-25^{o}C)
- Get the required null ELISA plate(s) ready
- Dilute goat anti-human IgM 1:1600 with carbonate buffer to make "plate coat", (6.25 µl of goat Anti-human IgM + 10 ml of 0.018 M Carbonate buffer.)
- Dispense100 µl "plate coat" to each well.
- Incubate 2 hrs at 35^{0}C / over night at room temperature (18ºC to 32ºC) .

If the plate is not to be used, transfer the plate without removing the sensitizing solution to -20º C (-25ºC to -15ºC). Plates may be stored for up to 1 month with no apparent loss of activity.

DAY II

7.2 Antibody Steps

Bring the coated plate and samples at room temperature

Specimen Dilution

Dilute normal control serum, weak positive control and test sera to 1:100 with 1XPBS
(10ul serum + 990ul 1XPBS)
Dilute CSF to 1:10 with 1XPBS
(30ul CSF + 270ul 1XPBS)

(Properly shake the samples before dilution)
Prepare the required washing solution
Wash plates **(repeat 6 times)** with washing solution

Dispense 50 µl of diluted controls and test specimen into the appropriate duplicate wells (see test plate arrangement).

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Kit: Linbro/ Titertek

EIA microtitration plate
96 flat bottom wells 1.0 X 0.7 cm approx.
Well capacity: 0.35 ml approx.
CAT. NO. 76-381-04
ICN BIOMEDICALS INC.
AURORA, OH 44202

LOT NO. 8010013

Test Date:
Plate no.:

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

WPC=weak positive control NC=negative control

Performed by: ss Checked by:
Incubate overnight at 2 hr at room temperature (18ºC to 32ºC)

Document Prepared by:
Supriya Sharma
Microbiologist
Date:9 Jan 2010

Document Authorized by:
Dr Geeta Shakya
Director
Date:11 Jan 2010

Effective Date: 11 Jan 2010
Review Date: 12 Jan 2012

7.3 Antigen Step

Bring the antigen and 13.5% NHS at room temperature

Dilute JEV antigen to 1:125 by using 40 µl of JE antigen, adding to5 ml of 13.5% NHS (acetone extracted) in PBS; (enough for 1 plate)

- Wash plates 6 times with PBST by aspirating serum from 96 wells by using automated Washer.
- Tap the plate on layers of towel paper several times to dry the plate.
- Dispense 50 µl of JE antigen solution into each test well.
- Incubate overnight at 4ºC (2ºC to 8ºC) or at least 2 hr at room temperature (18ºC to 32ºC).

DAY III

7.4. Conjugate Step

Bring the required reagents at room temperature

Prepare 0.18M Citrate phosphate buffer pH 5.0±0.1

Citric acid ($C_6H_8O_7$)	3.5	gm
Sodium phosphate, dibasic, anhydrous (Na_2HPO_4)	5.1	gm
Add distilled water to make final volume to	1000	ml
Check pH, 5.0 ± 0.1		

Prepare 0.5% Bovine Albumin in 13.5% NHS (acetone extracted) in PBS

Bovine Albumin Fraction-V	0.25	gm
13.5% NHS (acetone extracted) in PBS	50	ml

(1: 525). For 1 plate; 3ml dil. BSA sol+ 5.45ul IgG-HRP. (Human anti-flavivirus IgG-HRP)

- Dilute Human anti-flavivirus IgG-HRP in 13.5% acetone extracted NHS containing 0.5% Bovine Albumin to the previously determined working dilution (i.e.1: 525). **(For 1 plate 3ml dil. BSA + 5.45ul IgG-HRP)**
- *Do not let undiluted conjugate warm to room temperature .Return vial of conjugate to freezer immediately after finish making dilution.*

- Wash plate as
- Dispense 30 µl/well diluted conjugate in each of the test wells. Incubate for 1 hr at 35ºC to 37ºC

7.5. Substrate Step

Prepare Substrate Solution

0.18 M citrate phosphate buffer	10ml
O-phenylenediamine	0.005gm
Freshly prepared(1 ml of 30% H_2O_2 and 9 ml D/W) 3% H_2O_2	33 µl

- Wash plate for 6 times with PBST and 2 more times with PBS.
- Dispense Substrate Solution 100µl/well to each of the test wells.
- Incubate at room temperature in the dark for 30 minutes. Check periodically the color of WPC wells by slightly opening the box and taking the plate out to observe (the color should be identical to Rainin yellow color 200 µl plastic micropipette tip)
- Stop reaction by adding 50 µl of 4M H_2SO_4 to each well. Cover the plate with Seal Plate before reading the plate.
- Read the result by in the ELISA reader at wavelength 492 nm against 630nm.

8.0 INTERPRETATION GUIDELINES

8.1 Calculating a binding index (BI):

$$BI = \frac{\text{OD (test sample) - OD (neg. control)}}{\text{OD (WPC) - OD (neg. control)}}$$

EIA Units (U) = BI X 100

The weak positive control (WPC) is defined as 100 units.

A value of ≥ 40 units is positive.

8.2 In the clinical setting of encephalitis, CSF with ≥ 40 units of anti-Japanese Encephalitis IgM is very suggestive of a diagnosis of "Japanese Encephalitis".

8.3 If CSF is not available, serum with ≥ 40 units of anti-Japanese Encephalitis IgM suggests a diagnosis of "Probable Japanese Encephalitis."

8.4 Conditions to complete repeat testing

8.4.1. When the OD of the weak positive control is not in the range of 0.25-0.55, repeat testing of the entire plate is recommended

8.4.2 When the OD of duplicated tests is differed by 2 times, individual specimen should be repeated.

9. QUALITY CONTROL

9.1.Each kit contains a Weak Positive Control (WPC) and Negative Control (NC).

Acceptable values are as follows:

Weak Positive Control Absorbance of 0.25 – 0.55

Negative Control Absorbance of <0.1

9.2.The test is invalid if absorbance readings of controls do not meet these specifications.

10. PROCEDURAL NOTES

10.1. Major sources of inter-assay variation are:

- Dilution of enzyme conjugated anti-flavivirus IgG;
- Duration of the chromogen-substrate reaction;
- Amount of anti-isotype antibody bound to the plate in the initial step (plate-coat or sensitization step).

Because samples and antigen are applied to wells in relative excess, only the plate-coat and enzyme conjugate are limiting, i.e. these defined the assay signal strength. This assay is specifically designed to utilize Flow laboratories "Linbro/Titertek" flat bottom 96 well plates (plates not

provided in kit; Flow cat no. 76-381-04). Other plates such as Dynatech Immulon series plates may be less satisfactory. To reduce variation in sensitization of plates, it is recommended that plates be sensitized in batches and stored at -20ºC (-25ºC to -15ºC). Plates may be kept at -20ºC (-25ºC to -15ºC) for up to one month with no apparent loss of anti-isotype antibody bound to the plastic.

10.2.Before actually testing specimens for anti-Japanese encephalitis IgM antibody, the assay dilution of the anti-flavivirus IgG-HRP conjugate must be determined. The assay dilution is that which yields an OD at 492 nm of approximately 0.40 + 0.15 when a 1:100 dilution of the "weak" positive standards are used as the test sera. In our experience, this should be rechecked bi-monthly. The conjugate, which is supplied in 50% glycerol, gave satisfactory results when diluted 1:350 at the time it was shipped, i.e. OD of the JE IgM weak positive standard was 0.44 and the OD of the negative control was 0.02. If kept unopened at -20º C (-25ºC to -15ºC), it is stable for at least 6 months.

10.3 The chromogen reaction of the weak positive control consistent with an OD of 0.40 is a yellow color identical to that of a Rainin 200 µl plastic micropipette tip. Depending on the ambient temperature of the laboratory and the activity of the conjugate, the chromogen-substrate incubation step may be needed from 20-30 minutes up to 45 minutes. Watching the color development in the weak positive control well can monitor this. It is important to ensure that the chromogen solution is at room temperature when it is applied to the plate.

10.4 Notes on setting up the 96-well plate:
When performing the test with clinical specimens, each serum or cerebrospinal fluid (CSF) specimen should be tested against Japanese Encephalitis antigen in duplicate wells and the results averaged. Do not use wells A1-2, A11-12, H1-2, H11-12. Reactions in these wells are not reproducible. The procedure is designed for an assay of 40 test specimens for JE IgM.

10.5 This assay is designed as a 2- day procedure, i.e. dispense test specimens in the afternoon of day 1, and complete the assay in a 4-hour period during day 2. However, it may be more convenient to extend the period of incubation for step 6.3 (addition of antigen). Please note that the assay may be done as a "short" or "long" test, depending on the length of step 7.2 and 7.3.

10.6 The coefficient of intra-assay variation is approximately 10%. In one assay, the weak positive standard should give an absorbance in the range of 0.25 to 0.55. Values outside of this range are

unacceptable. Too high an absorbance will decrease the sensitivity of the test if a cutoff of 40 units is utilized. Too low a positive standard will decrease the specificity of the test.

11. LIMITATIONS/INTERFERENCES

- Linbro EIA Microtitration Plate, 96 Flat bottom wells 1.0x0.7 cm CAT No. 76-381-04 (ICN Biomedical Inc.)
  (the other EIA plate such as Dynatech give high OD)
- Humidified Reaction Chamber should be of size 24 x 33 x 11 cm (can keep the box warm)
- The weak reaction could be enhanced by keeping the plates in reaction chamber and put in incubator and observe
  the color
- Washing system should be free from contamination, suggest rinsing by siphon.

12. REFERENCES

1. WHO Manual for the Laboratory diagnosis of Japanese Encephalitis (WHO), 2007

END OF DOCUMENT

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Standard Operating Procedure (SOP) No.	JE 009
Prepared Date:	9 Jan 2010
Effective Date:	11 Jan 2010
Total No. of copies:	2
Distribution:	National Measles Laboratory, NPHL,Teku-1 copy BP Koirala Institute of Health Sciences,Dharan-1copy
Review Period:	2 years

APPROVED BY		
Title	**Signature and Name**	**Date**
Document Author	…….. Supriya Sharma Microbiologist, NPHL	9 Jan 2010
Authorized by	…….. Dr Geeta Shakya Director, NPHL	11 Jan 2010

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: JE IgM ELISA using AFRIMS kits for CSF and serum samples

SOP CODE.: JE 008

1. OBJECTIVE: This standard operating procedure provides guideline for detection of IgM antibodies to Japanese Encephalitis in serum collected from Acute Encephalitis Syndrome (AES) patients.

2. SCOPE: Detection of IgM antibodies to Japanese Encephalitis in serum collected from Acute Encephalitis Syndrome (AES) patients can be done using Panbio JE-dengue IgM combo ELISA kits.

3. INTRODUCTION

The Panbio Japanese Encephalitis - Dengue IgM Combo ELISA is for the qualitative presumptive detection of IgM antibodies to Japanese encephalitis and dengue virus in serum in patients with clinical symptoms consistent with encephalitis or dengue fever. This assay is a serological aid to diagnosis of Japanese encephalitis or dengue infection and positive results should be confirmed by PRNT or current CDC guidelines.

Japanese encephalitis (JE) and dengue are mosquito borne viruses belonging to the family Flaviviridae and the serological complex of Japanese encephalitis. JE is a common cause of human viral encephalitis in temperate and tropical regions throughout the world. JE has been implicated in periodic outbreaks of encephalitis cases throughout Asia and the Western Pacific countries and hence poses a major public health problem. JE infection has an incubation period of 6-12 days and results in an acute illness with symptoms including fever, convulsions and a depressed level of consciousness and coma. With JE infection there is a high fatality rate and high prevalence of neurological sequelae in those who survive the acute illness. Though JE infections can have serious side effects there is a high ratio of asymptomatic to symptomatic infections. JE is transmitted to humans by infected mosquitoes and is maintained in infected invertebrate reservoirs. In domestic animals such as swine and horses, JE infection causes still births, encephalitis and occasionally death. Dengue is found in large areas of the tropics and sub-tropics. Transmission is by mosquito, principally *Aedes aegypti* and *Aedes albopictus*. Dengue virus infection causes a spectrum of clinical manifestations ranging from unapparent to fatal haemorrhagic disease. Classic dengue or breakbone fever is characterized by the sudden onset of fever, intense headache, myalgia, arthralgia and rash. A diphasic febrile course is

common, as is insomnia and anorexia with loss of taste or bitter taste. Dengue haemorrhagic fever and dengue shock syndrome are severe complications often associated with a second serotype infection. Detection of IgM antibodies to dengue virus by ELISA is a valuable procedure, particularly in second and subsequent infections where the occurrence of complications is high. Serum IgM antibodies can be detected from dengue patients as early as three to five days after the onset of fever and generally persist for 30-90 days, although detectable levels may be present eight months post-infection.

4. PRINCIPLE: Each serum is added to 2 microwells. Serum antibodies of IgM class, when present, combine with anti-human IgM antibodies attached to the polystyrene surface of the microwell test strips. Concentrated Japanese encephalitis and dengue 1-4 antigens are diluted separately to the correct working volume with Antigen Diluent. The antigens are produced using an insect cell expression system and immunopurified utilizing a specific Tracer. An equal volume of the HRP-conjugated Monoclonal Antibody (MAb) is added to each diluted antigen, which allows the formation of antigen-MAb complexes. Residual serum is removed by washing and complexed antigen- MAb is added to the microwells: JE-MAb complex to one well and Dengue-MAb complex to the other well of the sample duplicates. After incubation, the microwells are washed and a colourless substrate system, tetramethylbenzidine/hydrogen peroxide (TMB Chromogen) is added. The substrate is hydrolyzed by the enzyme and the chromogen changes to a blue coour. After stopping the reaction with acid, the TMB becomes yellow. Colour development is indicative of the presence of the respective anti-flavivirus IgM antibodies in the test sample.

5.REQUIREMENTS:

MATERIALS PROVIDED in the kit

1. Anti-human IgM Coated Microwells - (12x8 wells) Microwells are coated with anti-human IgM antibodies. Ready for use. Unused microwells should be resealed immediately and stored in the presence of desiccant. Stable at 2-8ºC until expiry.
2. Japanese Encephalitis Antigen (Recombinant) - One Purplecapped vial, 150 µL concentrated JE viral antigen. Unused diluted antigen must be discarded. Concentrated antigen is stable at 2-8ºC until expiry.
3. Dengue 1-4 Antigens (Recombinant) - One Clear-capped vial, 150 µL (Blue) concentrated dengue viral antigens 1, 2, 3 and Unused diluted antigen must be discarded. Concentrated antigen is stable at 2-8ºC until expiry.

4. Wash Buffer (20x) - One bottle, 60 mL of 20x concentrate of phosphate buffered saline (pH 7.2-7.6) with Tween 20 and preservative (0.1% ProclinTM). Crystallisation may occur at low temperatures. To correct, incubate at 37ºC until clear. Mix well. Dilute one part Wash Buffer with 19 parts of distilled water. Diluted buffer may be stored for one week at 2-25ºC.
5. Sample Diluent - Two bottles, 50 mL (Pink). Ready for use. Tris buffered saline (pH 7.2-7.6) with preservatives (0.1% Proclin) and additives. Stable at 2-8ºC until expiry.
6. Antigen Diluent – One Blue-capped bottle, 50 mL. Ready for use. Phosphate Buffer containing preservatives (0.1% ProclinTM and 0.005% gentamycin). Stable at 2-8ºC until expiry.
7. HRP Conjugated Monoclonal Antibody Tracer - One bottle, 7 mL (Yellow). Ready for use. Horseradish peroxidase conjugated monoclonal antibody tracer with preservative (0.1% Proclin™) and protein stabilisers. Stable at 2-8ºC until expiry.
8. TMB Chromogen (TMB) - One bottle, 15 mL. Ready for use. A mixture of 3,3',5,5'-tetramethylbenzidine and hydrogen peroxide in a citric acid citrate buffer (pH 3.5-3.8). Stable at 2-8ºC until expiry.
9. Positive Control - One Black-capped vial, 200 µL human serum (contains 0.1% sodium azide and 0.005% gentamycin sulphate). Stable at 2-8ºC until expiry.
10. Dengue Calibrator – One Orange-capped vial, 400 µL human serum (contains 0.1% sodium azide and 0.005% gentamycin sulphate). Stable at 2-8ºC until expiry.
11. JE Calibrator - One Red-capped vial, 400 µL human serum (contains 0.1% sodium azide and 0.005% gentamycin sulphate). Stable at 2-8ºC until expiry.
12. Negative Control - One White-capped vial, 200 µL human serum (contains 0.1% sodium azide and 0.005% gentamycin sulphate). Stable at 2-8ºC until expiry.
13. Stop Solution – One Red-capped bottle, 15 mL. Ready for use. 1M Phosphoric acid. Stable at 2-25ºC until expiry.

ADDITIONAL MATERIALS REQUIRED BUT NOT PROVIDED

Equipment	Consumables	Registers
Micropipettes Incubator ELISA plate washer ELISA reader	Distilled water Tissue Paper Yellow Box, Ice packs Gloves, Marker Autoclavable Discarding Bin, 1% Hypochlorite solution	Japanese Encephalitis register Requisition File Acknowledgement slip

6. RESPONSIBILITIES:
It is the responsibility of the laboratory personnel to properly test the specimens and report the result accordingly to NPHL, Teku and WHO-IPD,Chakupat.

7. DESIGNATED AREA: National Measles/JE Laboratory, Teku, Kathmandu, Nepal

8. PROCEDURE:

1) Bring all the samples and the reagents required for ELISA to room temperature.

2) Dilute 10µl of antigen into 2.5ml of antigen diluent in 15ml tubes (1/250 dilution) . Prepare dilutions separately for JE and DENGUE antigen.

3) Mix required amount of JE and DENGUE antigen separately with an equal amount of Mab tracer in 15ml tubes. Leave it at room temperature until use. Discard any unused antigen.

4) Remove required number of wells and insert them into the frame

5) Dilute positive, negative controls, JE and DENGUE calibrators and serum samples as follows using 1.5ml Microcentifuge tubes : 10µl of control / sample in 1000µl of sample diluent (1/100 dilution)

6) Transfer 100µl of diluted controls and serum samples into respective wells and enter accordingly in the template as below:

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8
TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**
SOP CODE.: JE 008

Name of the Assay : Panbio JE & DEN IgM combo ELISA PlateID…….
Batch No. of the Kit…….
Date……..
Lot No. of the Kit……. Assay Performed by:…….
Expiry date of Kit…….. Name of Lab:…….

	1	2	3	4	5	6	7	8	9	10	11	12
	JE	**DEN**	**JE**	**DEN**	**JE**	**DEN**	**JE**	**DEN**	**JE**	**DEN**	**JE**	**DEN**
A	**Negative control**	**Negative control**	**S4**	**S4**								
B	**Positive control**	**Positive control**	**S5**	**S5**								
C	**JE Cal**	**Den Cal**	**S6**	**S6**								
D	**JE Cal**	**Den Cal**										
E	**JE Cal**	**Den Cal**										
F	**S1**	**S1**										
G	**S2**	**S2**										
H	**S3**	**S3**										

7) Cover the plate with aluminum foil and incubate at 37ºC for 1 hour.

8) Preparation of wash buffer :

Dilute 1 part of wash buffer concentrate in 19 parts of distilled water.

9) Wash the plate 6 times with the diluted wash buffer. Tap the plate on tissue paper after last wash.

10) Add 100µl of JE antigen – Mab complex and DENGUE antigen – Mab complex prepared earlier into the respective wells.

11) Cover the plate and incubate for 1 hour at 37ºC.

12) Wash the plate 6 times with the diluted wash buffer. Tap the plate on tissue paper after last wash.

13) Add 100µl of TMB into each well . Cover the plate.

14) Incubate for 10 minutes in dark at room temperature.

15) Add 100µl of stop solution into each well.

16) Read the absorbance at a wavelength of 450nm with a reference filter of 600 – 650 nm within 30 minutes.

CALCULATIONS:

1) Calculate the average OD of the calibrators added in triplicate.

2) Calculate the cut-off value :

Cut-off value = Mean absorbance of the calibrators **X** calibration factor (batch specific)

3) Calculate index value of the sample :

$$\text{Index value} = \frac{\text{OD of sample}}{\text{Cut –off value}}$$

4) Calulate the Panbio units of the sample:

Panbio units = Index value of sample **X** 10

INTERPRETATION OF PANBIO UNITS :

JE panbio units	JE IgM result	Dengue Panbio units	Dengue IgM result	Interpretation
< 9	Neg	< 9	Neg	No detectable IgM antibody.The result does not rule out the infection. An additional sample should be collected in 7-14 days and the test carried out again if early infection is suspected.
< 9	Neg	9 -11	Eqv	Samples should be re-tested
9-11	Eqv	< 9	Neg	Samples should be re-tested
9-11	Eqv	9-11	Eqv	Samples should be re-tested
< 9	Neg	>11	Pos	Calculate JE/ Dengue ratio : **Ratio = JE Panbio units / Dengue Panbio units**. Interpretation of JE / Dengue ratio : 1) > = 1 Presence of detectable IgM antibody presumptive infection with JE 2) < 1 Presence of detectable IgM antibody presumptive infection with Dengue
9-11	Eqv	>11	Pos	
>11	pos	<9	Neg	
>11	pos	9-11	Eqv	
> 11	pos	> 11	Pos	

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Document Prepared by:
Supriya Sharma
Microbiologist
Date:9 Jan 2010

Document Authorized by:
Dr Geeta Shakya
Director
Date:11 Jan 2010

Effective Date: 11 Jan 2010
Review Date: 12 Jan 2012

9. TEST LIMITATIONS

1. The clinical diagnosis must be interpreted with clinical signs and symptoms of the patient. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
2. Population seroepidemiology may vary over time in different geographical regions. Consequently, the cut-off may require adjustment based on local studies.
3. Screening of the general population should not be performed. The positive predictive value depends on the likelihood of the virus being present. Testing should only be performed on patients with clinical symptoms consistent with encephalitis or dengue fever.
4. Heterophilic antibodies are a well-recognised cause of interference in immunoassays. These antibodies to animal IgG may cross-react with reagent antibodies and generate a false positive signal. This must be excluded before confirmation of diagnosis.
5. The assay performance characteristics have not been established for visual result determination.
6. This assay employs insect-expressed proteins. The crossreactivity or interference of human anti-insect antibodies is unknown with the assay's results.
7. All sera demonstrating a positive result by the Panbio Japanese Encephalitis - Dengue IgM Combo ELISA should be referred to a reference laboratory for confirmation of positivity and epidemiological recording.
8. Serological cross-reactivity across the flavivirus group is common. Other flavivirus diseases must be excluded before confirmation of diagnosis. In-house studies with sera from infected Thai and Vietnamese patients have shown that the use of the Panbio Japanese Encephalitis - Dengue IgM Combo ELISA can be used to distinguish between JE and dengue infections.
9. Secondary dengue infections that are characterised by a sharp increase in anti-dengue IgG level with no apparent IgM increase will not be detected by the assay and require additional testing.

10. REFERENCES

1. Kit manual JAPANESE ENCEPHALITIS - DENGUE IgM COMBO ELISA TEST (E-JED01C)
2. WHO Manual for the Laboratory diagnosis of Japanese Encephalitis (WHO), 2007

END OF DOCUMENT

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Standard Operating Procedure (SOP) No.	JE 010
Prepared Date:	9 Jan 2010
Effective Date:	11 Jan 2010
Total No. of copies:	2
Distribution:	National Measles/JE Laboratory, NPHL,Teku-1 copy BP Koirala Institute of Health Sciences,Dharan-1copy
Review Period:	2 years

APPROVED BY		
Title	**Signature and Name**	**Date**
Document Author	…….. Supriya Sharma Microbiologist, NPHL	9 Jan 2010
Authorized by	…….. Dr Geeta Shakya Director, NPHL	11 Jan 2010

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

1. OBJECTIVE: This standard operating procedure provides guideline for detection of IgM antibodies to Japanese Encephalitis in serum and CSF collected from Acute Encephalitis Syndrome (AES) patients.

2. SCOPE: Detection of IgM antibodies to Japanese Encephalitis in serum collected from Acute Encephalitis Syndrome (AES) patients can be done using Xcyton JEV CheX ELISA kits.

3. INTRODUCTION

JEV CheX is a semiquantitive IgM capture ELISA for the detection of IgM antibodies against Japanese Encephalitis virus in human CSF and serum. This kit is developed in collaboration with NIMHANS. JEV CheX is IgM capture ELISA, which eliminates competition with IgG and thus helps in diagnosing recent infection. It uses cell culture antigen; avoids preparation of antigen in suckling mice. JEV CheX is a stabilized kit and can be stored at 4^0C for 6 months unlike the conventional test which required storage of reagents at -70^0C. JEV CheX has an assay time of 3 hours when compared to the 36 hours of time taken by the conventional test conducted at public health institutes.

4. PRINCIPLE

JEV CheX uses anti-human IgM coated plates to capture IgM antibodies in the clinical samples. Unbound excess CSF or serum is washed out and the wells are incubated with JEV specific antigen. This antigen binds to JEV specific IgM, if any in the well. A biotinylated- MAb specific to JEV antigen is added after washing off the excess antigen. This monoclonal binds to the antien-IgM complex. Excess monoclonal is washed off and streptavidin peroxidase is added which binds to the biotinylated –monoclonal, if present in the well. A substrate is added after washing off excess streptavidin-peroxidase to develop colour reaction. After 10 min this reaction is stopped by the addition of stop solution and the intensity of the colour is read at 450nm using an ELISA reader.

5.REQUIREMENTS:

Equipment	**Consumables**	**Registers**
Micropipettes Incubator ELISA plate washer ELISA reader	Distilled Water, Pipette tips Tissue Paper,Gloves, Marker Yellow Box, Ice packs Autoclavable Discarding Bin, 1% Hypochlorite solution	Japanese Encephalitis register Requisition File Acknowledgement slip

6. RESPONSIBILITIES:
It is the responsibility of the laboratory personnel to properly test the specimens and report the result accordingly to NPHL, Teku and WHO-IPD,Chakupat.

7. DESIGNATED AREA: National Measles/JE Laboratory, Teku, Kathmandu, Nepal

8. PROCEDURE:

1) Bring all the samples and the reagents required for ELISA to room temperature.
2) Take the required number of wells and insert them into the frame.
3) Add 100µl of diluent to all the wells.
4) Well A1 serves as blank.
5) Add 10µl of negative control into wells B1, C1, D1.
6) Add 10µl of positive control into wells E1, F1.
7) Add 10µl of weak positive control into wells G1, H1.
8) Pipette 10µl of each test CSF sample (5µl of each test serum sample) into respective well and mix thoroughly. Enter into the template accordingly.

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Government of Nepal
Ministry of Health and Population
Department of Health Services

National Public Health Laboratory

Teku, Kathmandu

NATIONAL MEASLES/JE LABORATORY
STANDARD OPERATING PROCEDURE-8

TITLE: **JE IgM ELISA using AFRIMS kits for CSF and serum samples**

SOP CODE.: JE 008

Name of the Assay : Xcyton JEV Chex ELISA :……..

PlateID

Batch No. of the Kit…….. :……

Date

Lot No. of the Kit…….. by:……

Assay Performed

Expiry date of Kit…….. :……..

Name of Lab

	1	2	3	4	5	6	7	8	9	10	11	12
	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID	Sample ID
A	Blank	Weak Positive control serum										
B	Negative Control	Weak Positive control serum										
C	Negative Control											
D	Negative Control											
E	Positive control											
F	Positive control											
G	Weak Positive control CSF											
H	Weak Positive control CSF											

9) Cover the plate with the aluminum foil and incubate for 1 hour at 37ºC.

10) Preparation of wash buffer:

Dilute 50ml of wash buffer concentrate in 1000ml of distilled water.

11) Wash the plate 5 times with the diluted wash buffer.

12) Add 100µl of ready to use antigen provided in the kit

13) Incubate for 1 hour at 37ºC.

14) Wash the plate 5 times with the diluted wash buffer.

15) Add 100µl of diluted biotinylated antibody to each well. **(For dilution refer Table 1)**

Table 1: Dilution of biiotinylated antibody

Number of wells used	Volume of 10x biotinylated monoclonal antibody to be used(in µl)	Volume of diluent(in ml)
8	100	0.9
16	200	1.8
24	300	2.7
32	400	3.6
40	500	4.5
48	600	5.4
56	700	6.3
64	800	7.2
72	900	8.1
80	1000	9.0
88	1100	9.9
96	1200	10.8

16) Incubate for 30 minutes at 37ºC.

17) Wash the plate 5 times with the diluted wash buffer.

18) Add 100µl of diluted streptavidin – peroxidase conjugate to each well.
(For dilution refer Table 2)

Table 2: Reconsitution of streptavidin peroxidase conjugate and its dilution.

1. Add 150 µl of streptavidin peroxidase diluent to sreptavidin peroxidase concentrate(100x concentrate) . Mix well.
2. Refer to Table below

Number of Wells used	Volume of 100x reconsituted sreptavidin Peroxidase conjugate(in µl)	Volume of Diluent(in ml)
8	10	1.0
16	20	2.0
24	30	3.0
32	40	4.0
40	50	5.0
48	60	6.0
56	70	7.0
64	80	8.0
72	90	9.0
80	100	10.0
88	110	11.0
96	120	12.0

19) Incubate at room temperature for 15 minutes.

20) Wash the plate 5 times with the diluted wash buffer .

21) Add 100µl of diluted substrate into each well.**(For dilution refer Table 3)**

Table 3:Dilution of substrate

1. Prepare the solution just before use. Dilute the substrate in the 15 ml tube provided in the kit.
2. Refer to Table below

Number of wells used	Volume of 20x substrate(in µl)	Volume of Diluent(in ml)
8	50	1.0
16	100	2.0
24	150	3.0
32	200	4.0
40	250	5.0
48	300	6.0
56	350	7.0
64	400	8.0
72	450	9.0
80	500	10.0
88	550	11.0
96	600	12.0

22) Incubate for 10 minutes in dark at room temperature.

23) Add 100µl of stop solution to each well.

24) Read the absorbance at 450nm with 630nm as reference filter within 60 minutes.

CALCULATIONS:

Calculate the ELISA units using the formula provided below:

$$\text{ELISA units} = \frac{\text{Test OD value} - \text{Mean OD of negative control}}{\text{Mean OD of weak positive control} - \text{Mean OD of negative control}} \times 100$$

VALIDITY CRITERIA :

1) Mean OD of Negative control should not exceed 0.200 (i.e. < 0.200)
2) OD of Positive control should be not less than 0.800(i.e > 0.800)
3) OD of Weak positive control should be between 0.200 – 0.800

INTERPRETATION OF RESULTS :

ELISA units	Interpretation
> 30	Negative
30 - 99	Flavivirus positive
> = 100	JE positive

9. REFERENCES

1. Kit manual Xcyton JEV CheX ELISA kit
2. WHO Manual for the Laboratory diagnosis of Japanese Encephalitis (WHO), 2007

END OF DOCUMENT