Virus IgM antibody detection

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ABSTRACT

Two JEV IgM antibody detection kits were used to test serum and CSF samples.the Beixi Capture ELISA 24 Test ELISA kit (REVI-001M,LOT:1807-1;Shanghai B&C ,China)andthe JE Detect IgM Antibody Capture ELISA Kit(JEMS-1,LOT:XC1285,InBios International,Inc,Seattle,WA,USA).The West Nile Virus (WNV) IgM antibody was detected by the Capture DxSelect Enzyme-linked Immunosorbent Assay ELISA Kit(EL0300M,LOT:1990N,Focus,Saettle,WA,USA).The experimental procedures and interpretation of results were carried out in accordance with the manufacturer's instructions .

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GUIDELINES

The WNV test kit can only test serum samples.

MATERIALS TEXT

the Beixi Capture ELISA 24 Test ELISA kit ;the JE Detect IgM Antibody Capture ELISA Kit ;the Capture DxSelect Enzyme-linked Immunosorbent Assay ELISA Kit ; serum samples and CSF samples.

BEFORE STARTING

samples should be kept in room temperture before the test start.

Beixi kit

1 1. After the kit has equilibrated to room temperature, unpack the microplates and remove the required amount of strips and place them in the holder. The remaining strips should be re-placed in the packaging bag for sealing and stored at 2-8°C.

2 2. Cerebrospinal fluid samples were diluted 1:10 with normal saline and serum samples were diluted 1:100 with normal saline. Add 50 ul of diluted serum or cerebrospinal fluid specimen to the corresponding well. Set each well of negative and positive quality control, and add 50 ul negative and positive quality control, respectively (negative and positive quality control do not need to be diluted). Set another well as blank control.

3 3. Seal the plate and incubate at 37°C for 1 hour.

4 4. Discard the liquid in microwells, fill each well with washing solution, allow to stand for 30 seconds and shake to dryness, repeat for 4 times. After the last plate washing, pat the residual liquid in the wells on the absorbent paper.

5 5. 50 ul of JE antigen-enzyme conjugate mixture was added to each well, blank wells were not added, and incubated at 37°C for 1 hour.

6 6. Discard the liquid in microwells, fill each well with washing solution, allow to stand for 30 seconds and shake to dryness, repeat for 4 times. After the last plate washing, pat the residual liquid in the wells on the absorbent paper.

7 7. Add 1 drop of chromogenic solution A and 1 drop of chromogenic solution B to each well, mix well and allow to react at 37°C, protected from light for 10 minutes.

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If determined visually, allow the reaction to proceed for 10 minutes at room temperature (20-28°C) in the dark after adding the chromogen.

8 8. One drop of stop solution was added to each well, and the OD value of each well was measured at 450 nm.

the JE Detect IgM Antibody Capture ELISA Kit

9 1, all reagent components and samples were equilibrated to room temperature (25 ° C). Reagents and samples were mixed prior to the experiment. Positive and negative quality controls must be tested in duplicate, and JE reactive antigen (JERA) and normal cell antigen (NCA) should be tested for each serum and cerebrospinal fluid sample.

10 2, the serum and quality control were diluted 100-fold with the sample diluent provided with the kit. Dilute with 1.5 ml EP tube, and at least 4 ul serum, positive control and negative control are required for dilution. For example: 4 ul serum + 396 ul diluent.

11 3, 50 ul of diluted serum, negative control, and positive control were added to the corresponding wells.

12 4, plates were mounted with plate sealant plywood only on the open microwell surface and incubated for 1 h at 37 ° C.

13 5, after incubation, the plate was washed six times with 1X washing solution on a plate washer, 300 ul per well each time

14 6, 50 ul JERA was added to one set of specimens to be tested, and 50 ul NCA was added to another set of identical specimens to be tested.

15 7, plates were mounted with plate sealant plywood only on the open microwell surface and incubated for 1 h at 37 ° C.

16 8, after incubation, the plate was washed six times with 1X washing solution on a plate washer, 300 ul per well each time, and at the end of the last plate washing, the residual droplets in the wells were patted dry on absorbent pape

17 9, 50 ul of HRPO horseradish peroxidase-labeled enzyme conjugate was added to each well.

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18 10, plates were mounted on the open microwell surface only with plate sealing plywood and incubated for 1 h at 37 ° C.

19 11, after incubation, the plate was washed six times with 1X washing solution on a plate washer, 300 ul per well each time, and at the end of the last plate washing, the residual droplets in the wells were patted dry on absorbent paper.

20 12, 150 ul of EnWash solution was added to each well.

21 13, incubated at room temperature (without covering) for 5 min.

22 14, after incubation, the plate was washed six times with 1X washing solution on a plate washer, 300 ul per well each time, and at the end of the last plate washing, the residual droplets in the wells were patted dry on absorbent paper.

23 15, 75 ul of TMB substrate solution was added to each well using a multichannel pipette.

24 16, incubated at room temperature in the dark for 10 min.

25 17, 50 ul of stop solution was added to each well and incubated at room temperature for 1 min.

26 18, after incubation, the OD value was measured at 450 nm.

WNV test kit

27 1. diluted sample: patient serum and control serum: dilute with sample diluent at a ratio of 1:101 (such as 10 μL serum + 1000 μL sample diluent).

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28 2. add 1X wash buffer and allow to stand for 5 minutes and discard the liquid.

29 3. add 100 µL of sample and incubate for 60 minutes and discard the liquid.

30 4. wash the plate three times.

31 5. add 100 µL of antigen, incubate for 120 minutes, and discard the liquid.

32 6. wash the plate three times.

33 7. add 100 µL of IgM Conjugate and incubate for 30 minutes, discarding the liquid.
8. Wash the plate three times.

34 9. add 100 µL of enzyme substrate (Substrate Reagent) and incubate for 10 minutes.
10. add 100 µL of Stop Reagent and read at 450 nm

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