PRINCIPLE OF THE TEST

In this EV71 IgM Dot-ELISA, nitrocelulose membrane is pre-coated with antibodies directed to human immunoglobulin M proteins (anti-μ chain). The patient’s sample is added and it filtrates through the membrane. During filtration, any IgM antibodies will be captured onto the membrane. After washing out all the other substances of the sample and in particular IgG-class antibodies, purified EV71 antigen and EV71 antibodies conjugated to the enzyme horseradish peroxidase (HRP-conjugate) are added into to the membrane. These antigens and antibodies will bind to any, previously captured onto the membrane anti-IgM EV71 antibodies. Substrate is then added and allowed to incubate for two minutes, resulting in a blue ellipse developing on the membrane indicating a positive test. The test has integrated quality control: a blue dot will always appear indicating the validity of the test.

INTENDED USE

This EV-71 IgM DOT-ELISA is an enzyme-linked immuno-filtration method for qualitative determination of IgM-class antibodies to human enterovirus-71 (EV71) in serum or plasma samples. The assay is intended to be used in clinical laboratories for early diagnosis and management of patients related to infection with EV71.

GENERAL DESCRIPTION

Human Enterovirus 71 (EV71), the newest member of Enteroviridae, is notable for its etiological role in epidemics of severe neurological diseases in children. It appears to be emerging as an important virulent neurotropic enterovirus in the upcoming era of poliomyelitis eradication. The illness usually peaks in June or July. EV71 infection may be asymptomatic or may cause diarrhea and rashes. EV71 one of the major causative agents for hand, foot and mouth disease (HFMD), is sometimes associated with severe central nervous system diseases. Direct detection of virus is the mainstay of diagnosis. EV 71 can be isolated from throat and stool specimens, as well as from skin vesicle fluid. PCR testing provides generally greater sensitivity than culture for throat and stool specimens, and viral RNA has also been detected in vesicular fluid, blood and urine. EV 71-specific serological assays, including tests specific for IgM antibody, have also been developed to assist for early and easier diagnosis of the disease.

STORAGE AND STABILITY

The components of the kit will remain stable up until the expiration date indicated on the label and package when stored between 2-8°C. DO NOT FREEZE. To assure maximum performance of this EV71 IgM Dot-ELISA during storage, protect the reagents from contamination with microorganisms or chemicals.

SPECIMEN COLLECTION AND HANDLING

Specimen collection: Samples are collected by following standard laboratory practices for collection of serum or plasma samples. It is recommended that samples that will not be tested within 24 hours after collection should be stored frozen at -80°C.

REAGENTS PROVIDED

Six tests/kit
1. 6 x EV71 IgM Immuno-filtration Devices in 2 aluminium pouchs with desiccant. The device has an integrated sample flow controller
2. Sample treatment solution: 1 bottle of 6ml
3. Sample treatment bottles: 6 bottle
4. Antigen reagent: 1 dropper bottle of 1.8ml
5. HRP-conjugate: 1 dropper bottle of 1.8ml. Ready for use as supplied
6. Wash Buffer: 1 dropper bottle of 6ml. Ready for use as supplied
7. Chromogen Solution: 1 bottle of 1ml. Ready for use as supplied
8. Stop Solution: 1 dropper bottle of 1ml. Ready for use as supplied
9. Instruction: 1 copy.
10. Materials required but not provided, clock or timer, disposable pipettes, biohazard waste container, positive/negative controls.
ASSAY PROCEDURE

Allow the reagents to stabilize at room temperature (approximately 30 minutes). Place the test devices in the designated area of the workstation.

1. Reagent And Sample Preparation

Sample preparation: Unscrew the cap of the sample treatment bottle and place it on a flat surface. Pipette 8ul of undiluted specimen into the bottle, and add 30 drops (about 800uL) of sample treatment solution buffer into the sample treatment bottle. Then TIGHTLY screw the cap of the sample treatment bottle and mix thoroughly for several times. For already diluted samples, use 600ul sample with 12 drops (about 300uL) of treatment solution.

2. Assay Steps

Adding sample: Point the dropper of the sample treatment bottle at the centre of the sample well and slowly add all of the pre-treated sample into the sample well (to prevent bubbles formation). Wait approximately 15-20 minutes till the sample fully filtrates through the membrane. After the sample is completely absorbed, remove the Flow Controller. Discard as biohazard.

Adding virus antigen and HRP-conjugate: Add 5 drops (about 250uL) of the provided virus antigen onto the reaction membrane and wait till reacts (about 2min.). Then add 5 drops (about 250uL) of the provided HRP-conjugate onto the reaction membrane and wait till reacts (about 2min.) then proceed to the next step immediately.

Washing: Wash the membrane 2 times with the provided Washing Buffer. Each time, add 12 drops (Approx 350ul) washing buffer. After each washing cycle, ensure the Washing Buffer has completely filtered through the membrane before proceeding to the next cycle.

Color Development: After the final washing cycle, add 2 drops (100ul) of Chromogen Solution onto the reaction membrane. Wait 2 minutes after the solution completely filters through the membrane and then add 1 drop (about 50uL) of Stop Solution onto the reaction membrane. Read the results within 3-5 minutes.

EVALUATION

Read the results in a well-lighted area within 3-5 minutes. Record the test results.

Positive Test (antigen present): In addition to the blue dot in the Control Zone, a distinctive blue ellipse will appear at the Test Zone, indicating that IgM antibodies to EV71 have been detected using this Dot-ELISA Test.

Negative Test (no antigen detected): Only the blue dot appears in the Control Zone, indicating that no IgM antibodies to EV71 have been detected with this Dot-ELISA Test. However this does not exclude the possibility of infection.

Quality Control: A blue dot will always appear in the Control Zone. If no blue dot appears in the Control Zone, the test is invalid; discard the test and repeat with a new sample and new device.

The positive result obtained with this test alone cannot be the final diagnosis of infection with Ev71. Any positive result must be interpreted in conjunction with other laboratory testing results and the result of a confirmative assay. Follow-up and supplementary testing with other analytical systems are required to confirm any positive results.

REFERENCES