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User's Manual

Human Anti-Crimean/Congo Hemorrhagic Fever Virus (CCHFV) IgM ELISA Kit

REF **DEIA-CL017**

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This product is for research use only and is not intended for diagnostic use.

For illustrative purposes only. To perform the assay the instructions for use provided with the kit have to be used.

Creative Diagnostics

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PRODUCT INFORMATION

Intended Use

The Human Anti-Crimean-Congo Hemorrhagic Fever Virus (CCHFV) IgM ELISA Kit detects and quantifies CCHFV -specific IgM in human serum or plasma of vaccinated, immunized and/or infected hosts. This immunoassay is suitable for:

_ Determining immune status relative to non- immune controls;

_ Assessing efficacy of vaccines, including dosage, adjuvantcy, route of immunization and timing;

_ Qualifying and standardizing vaccine batches & protocols.

General Description

Crimean-Congo hemorrhagic fever (CCHF) is a widespread tick-borne viral disease, a zoonosis of domestic animals and wild animals, that may affect humans. The pathogenic virus, especially common in East and West Africa, is a member of the Bunyaviridae family of RNA viruses. Clinical disease is rare in infected mammals, but is commonly severe in infected humans, with a 30% mortality rate. Outbreaks of illness are usually attributable to handling infected animals or humans. CCHF is distributed throughout Eastern Europe, the Mediterranean, northwestern China, central Asia Africa, the Middle East, and the Indian subcontinent.

The virus genome is circular, ambisense RNA in three parts - Small (S), Middle (M) and Large (L). The L segment encodes the RNA polymerase; the M segment encodes the envelope proteins (Gc and Gn); and the S segment encodes the nucleocapsid protein. The envelope protein is initially translated as a glycoprotein precursor which is then cleaved into two smaller proteins. Based on the sequence data seven genotypes have been recognized: Africa 1 (Senegal), Africa 2 (Democratic Republic of the Congo and South Africa), Africa 3 (southern and western Africa), Europe 1 (Albania, Bulgaria, Kosovo, Russia and Turkey), Europe 2 (Greece) Asia 1 (the Middle East, Iran and Pakistan) and Asia 2 (China, Kazakhstan, Tajikistan and Uzbekistan).

Vaccines: A Turkish research team led by Refik Saydam Health Institute has developed treatment- serum derived from blood of several CCHF-patients, which have been proven to be %90 effective in CCHF patients. The vaccine is pending for FDA approval.

ADI has cloned, expressed and purified CCHFV nucleoprotein (482-aa, ~55 kDa) that is being used as a candidate for newer subunit vaccine for CCHF.

Principles of Testing

The Human Anti-CCHFV IgM ELISA kit is based on the binding of human anti-CCHFV in samples to CCHFV nucleoprotein toxin immobilized on the microwells, and anti- CCHFV IgM antibody is detected by anti-Human IgM-specific antibody conjugated to HRP (horseradish peroxidase) enzyme. After a washing step, chromogenic substrate (TMB) is added and color is developed by the enzymatic reaction of HRP on the substrate, which is directly proportional to the amount of anti-CCHFV IgM present in the sample. Stopping Solution is added to terminate the reaction, and absorbance at 450nm is then measured using an ELISA microwell reader. The activity of antibody in samples is determined relative to anti-CCHFV calibrators.

Reagents And Materials Provided

Ready For Use: Store as indicated on labels.

CCHFV coated Strip Plate: 8- well strips (12), Coated with recombinant CCHFV NP, and post-coated with stabilizers.

Anti-CCHFV Calibrators: 0.65 ml/vial, Four (4) vials (1U/ml, 2.5 U/ml, 5 U/ml, 10 U/ml), each containing anti-CCHFV antibodies; in buffer with antimicrobial as stabilizers.

TMB Substrate: 12 ml, Chromogenic substrate for HRP containing TMB and peroxide.

Stop Solution: 12 ml, Dilute sulfuric acid.

The microtiter well plate and all other reagents, if unopened, are stable at 2-8°C until the expiration date printed on the box label. Stabilities of the working solutions are indicated under Reagent Preparation.

To Be Reconstituted: Store as indicated.

Wash Solution Concentrate (100x): 10ml, Dilute the entire volume 10ml + 990ml with distilled or deionized water into a clean stock bottle. Label as Working Wash Solution and store at ambient temp. until kit is used entirely.

Sample Diluent Concentrate (20x): 10ml, Dilute 0.5ml + 9.5ml with distilled or deionized water as needed for HRP Conjugate and Sample Dilution. Label as Working Sample Diluent and store at 2-8°C until the kit lot expires or is used up.

Anti-Human IgM - HRP Conjugate Concentrate (100x): 0.15ml, Peroxidase conjugated anti- Human IgM in buffer with protein, detergents and antimicrobial as stabilizers. Dilute fresh as needed; 10ul of concentrate to 1ml of Working Sample Diluent is sufficient for 1 strip. Use within the working day and discard. Return 100X to 2- 8°C storage.

Materials Required But Not Supplied

- Pipettors and pipettes that deliver 100ul and 1- 10ml. A multi-channel pipettor is recommended.

- Disposable glass or plastic 5-15ml tubes for diluting samples and Anti-Human IgM HRP Concentrate.

- Graduated cylinder to dilute Wash Concentrate; 0.2 to 1L.

- Distilled or deionized water to dilute reagent concentrates.

- Microwell plate reader at 450 nm wavelength and ELISA plate washer.

Assay Procedure

Sample Collection and Handling

For serum, collect blood by venipuncture, allow clotting, and separate the serum by centrifugation at room temperature. For other samples, including tissue culture media, clarify the sample by centrifugation and/or filtration prior to dilution in Sample Diluent.

Assay Design

Review Calculation of Results and Limits of the Assay (above) before proceeding:

- Select the proper sample dilutions accounting for expected potency of positives and minimizing non-specific binding (NSB) and other matrix effects; for example, net signal for non-immune samples should be <0.5 OD. This is usually 1/100 or greater dilution for human sera with normal levels of IgG and IgM.

- Run a Sample Diluent Blank. This signal is an indicator of proper assay performance, especially of washing efficacy, and is used for net OD calculations, if required

- Run a set of Calibrators. Calibrators validate that the assay was performed to specifications; results can be used to normalize between-assay variation for enhanced precision.

- Run a range of sample dilutions for expected higher positives that allows calculation of antibody Titer (when specific titer is at least 4-fold higher than non-immune). See Method B.

- The Calibrators that are used for quantitation, e.g., for between-assay normalization, should be run in duplicate. When determining titer from a dilution curve, singlicates can be run if more than two dilution points are used for titer calculations.

Plate Set-up

Bring all reagents to room temperature (18-30°C) equilibration (at least 30 minutes).

- Determine the number of wells for the assay run.

Duplicates are recommended, including 8 Calibrator wells and 2 wells for each sample and control to be assayed.

- Remove the appropriate number of microwell strips from the pouch and store unused strips in the pouch at 4°C.

- Add 200-300ul Working Wash Solution to each well and let stand for about 5 minutes. Aspirate or dump the liquid and pat dry on a paper towel before sample addition.

Assay Procedure

ALL STEPS ARE PERFORMED AT ROOM TEMPERATURE. After each reagent addition, gently tap the plate to mix the well contents prior to beginning incubation.

1. 1st Incubation [100ul - 60 min; 4 washes]

_ Add 100ul of calibrators, samples and controls each to pre-determined wells.

_ Tap the plate gently to mix reagents and incubate for 60 minutes.

_ Wash wells 4 times and pat dry on fresh paper towels. As an alternative, an automatic plate washer may be used. Improper washes may lead to falsely elevated signals and poor reproducibility.

2. 2nd Incubation [100ul - 30 min; 5 washes]

_ Add 100ul of diluted Anti-Human IgM HRP to each well.

_ Incubate for 30 minutes.

3. Substrate Incubation [100ul - 15 min]

_ Add 100ul TMB Substrate to each well. The liquid in the wells will begin to turn blue.

_ Incubate for 15 minutes in the dark, e.g., place in a drawer or closet.

Note: If ELISA reader does not register optical density (OD) above 2.0, incubate for less time, or read OD at 405-410 nm (results are valid).

4. Stop Step [Stop: 100ul]

_ Add 100ul of Stop Solution to each well.

_ Tap gently to mix. The enzyme reaction will stop; liquid in the wells will turn yellow.

5. Absorbance Reading

_ Read the plate at 450nm wavelength within 30 min.

_ Wash wells 5 times as in step 2.

Calculation

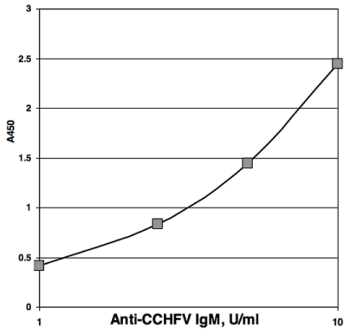
Consider several data reduction methods to best represent the relationships among experimental and control groups, to determine Positive Immune and Negative Non-immune. Here we present the use of a calibrator curve to determine the unknown sample values.

Use of a Calibrator Curve

When the dilution curves of samples are parallel to the Calibrator curve, the anti-CCHFV IgM activity (U/ml) may be determined as follows:

1. Calculate the mean OD of duplicate samples.
2. On graph paper plot the mean OD of the calibrators (y-axis) against the concn (U/ml) of anti- CCHFV (x-axis). Draw the best fit curve through these points to construct the calibrator curve.
3. The anti-CCHFV activity concentrations in unknown samples and controls can be determined by interpolation from the calibrator curve.
4. Multiply the values obtained for the samples by the dilution factor of each sample.
5. If an ELISA software is used, we recommend 4-PL curve fit.
6. Samples producing signals higher than the 10U/ml calibrator should be further diluted and re- assayed

Wells	Calibrators		A450 nm
A1,2	Negative Diluent Blank		0.08
B1,2	1 U/ml	Calibrator	0.41
C1,2	2.5 U/ml	Calibrator	0.83
D1,2	5 U/ml	Calibrator	1.40
E1,2	10 U/ml	Calibrator	2.44
F1,2	Sample	1:200	1.65
Sample Result: 5.8 U/ml x 200 dilution = 1160 U/ml			



Sensitivity

Assay Sensitivity

The CCHFV NP-coated plate and the anti-Human IgM HRP concentration are optimized to differentiate anti-CCHFV IgM from background (non-antibody) signal with human serum samples diluted 1:200.

Calibrator Values

The Calibrators are composed of dilutions of antibody to CCHFV NP. Values are assigned in arbitrary units.

Specificity

Purified recombinant (his tag; E.coli) CCHFV nucleoprotein (NP) is used to coat the microwells; thus, no other antibody specificity is detectable in the assay. The anti-Human IgM HRP conjugate specifically detects IgM, and will not react with IgG, IgA or IgE class antibodies.

Precautions

Calibrators, Sample Diluent, and Antibody HRP contain bromonitrodioxane (BND: 0.05%, w/v). Stop Solution contains diluted sulfuric acid. Follow good laboratory practices, and avoid ingestion or contact of any reagent with skin, eyes or mucous membranes. All reagents may be disposed of down a drain with copious amounts of water.

Limitations

To quantitate antibody activity from a calibrator curve (such as provided with the kit), the dilution curve of the samples must be parallel to the calibrator curve, to avoid different values being obtained from different regions of the curve. Antibodies that are not matched in CCHFV avidity will often have non-parallel dilution curves. In these cases, antibody activity is best expressed as a titer relative to a reference positive such as the 5 U/ml Calibrator, or another Calibrator in the kit.

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