Version 15-11/24

User's Manual

Hepatitis A-Virus (HAV) Antigen ELISA Kit

REF **DEIA-NB24-06**

Σ **96T**

RUO

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.

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PRODUCT INFORMATION

Intended Use

The HAV Antigen ELISA Kit is an enzyme immunoassay for research use for the identification of Hepatitis A virus in stool and cell culture.

General Description

A positive identification of Hepatitis A Virus (HAV) in human stool samples indicates a fresh and contagious infection with HAV. The passing begins about two weeks before the icteric phase of the disease and reached a peak after about one week before icterus. With the beginning of the icteric phase, the HAV passing drops steeply but HAV antigen could be found in the stool of some, not all, humans two weeks after onset of icterus.

The detection of HAV in specimen other than stool is also possible with the CD HAV Antigen ELISA Kit, for example in lysates of HAV infected cells or in culture supernatants. If neccessary the specimen must be concentrated before testing (ultrafiltration i.e.).

Specimen with high or low pH, high salt or detergens concentration should be dialysed against phosphate buffered saline (PBS).

Principles of Testing

The specimen are pipetted into wells of a microtiter plate previously coated with antibodies directed against HAV. The HAV antigen binds to the fixed antibody and after the incubation period of two hours at 37°C the plate is washed thoroughly. Bound HAV antigen is identified by conjugate addition (monoclonal anti-HAV, peroxidase conjugated) incubated for another two hours at 37°C. Excess conjugate is removed by washing and the substrate is added. After 30 minutes incubation at room temperature the reaction is terminated by adding stop solution. The blue colour of a positive reaction turns to yellow and is measured in a microplate reader at 450 nm. The intensity of the colour indicates the concentration of bound HAV antigen. To exclude possible false positive reactions, that may occure in stool, positive results are advised to be confirmed by the use of neutralising anti-HAV serum in a parallel or in a second measurement.

Reagents And Materials Provided

1. **Microtiter plate:** ready for use, coated with anti-HAV antigen-antibody. Wells are separately breakable. (8x12) wells
2. **Conjugate Concentrate (flask A):** 100-fold concentrate, contains the peroxidase conjugated mouse anti-HAV IgG. 1 x 150 µL
3. **Positive Control (flask B):** Hepatitis A-Virus antigen, inactivated, ready for use. 1 x 500 µL
4. **Neutralising serum (flask C):** anti-HAV-positive serum, 10× concentrated. 1 x 500 µL
5. **Dilution buffer (flask D):** ready for use, red colored. Dilution buffer for specimen und

conjugate, red coloured. 1 x 120 mL

6. **Substrate (flask E):** ready for use. 1 x 12 mL
7. **Stop solution (flask F):** ready for use, 0.2 M sulfuric acid. 1 x 12 mL

8. **Wash buffer (flask G):** 20-fold concentrated solution. 1 x 50 mL
9. **Sealing Tape:** for covering the microtiter plate. 2

Materials Required But Not Supplied

1. Distilled water for dilution of wash buffer
2. Centrifuge for preparation of stool specimen.
3. Incubator or water bath with an adaptor for microtiter plates.
4. Precision pipettes with disposable tips.
5. Microtiter Plate washer (recommended)
6. Microplate reader ("ELISA-Reader") with filter for 450 and ≥ 590 nm
7. Polyethylene PE/Polypropylene PP tubes for dilution of samples

Storage

All materials must be stored at 2 - 8°C in the dark. Unused microtiterplate stripes have to be stored airtight together with the desiccant bag at 2 - 8°C.

The shelf-life of the components after initial opening is guaranteed for four weeks.

Specimen Collection And Preparation

Prepare a 20 % (w/v) suspension of stool in Dilution Buffer (D). Centrifuge the suspension with at least 2400 g for 10 minutes at room temperature. The clear supernatant can be used in the test. If required repeat the centrifugation.

Supernatants of cell culture and cell lysates can be used directly. If required, they can be concentrated i.e. with ultracentrifugation.

Reagent Preparation

1. Bring all reagents to room temperature (20 - 25°C) before use.
2. Wash Buffer (G): Dilute the 20 x Wash Buffer 1:20 with distilled water. Attention: After dilution the Washing Buffer is only 4 weeks stable, please dilute only according to requirements.
3. Dilute the neutralising serum (C) for the confirmation of positive reactions 1:10 with dilution buffer (D). Dilute only the volume used in the test (50 µL per well). Diluted serum is stable for at least one week at 4°C.
4. Dilute the 100 x Conjugate Concentrate (A) 1:100 with Dilution Buffer (D). Dilute only the volume used in the test (100 µL per well). Diluted conjugate is stable for at least one week at 4°C.

Assay Procedure

Note

1. Incubation at room temperature means: 20 - 25°C.
2. Proper washing is of basic importance for a secure, reliable and precise performance of the test. Incomplete

washing is common and will adversely affect the test outcome. Possible consequences may be uncontrolled unspecific variations of measured optical densities, potentially leading to false results calculations of the examined samples. Effects like high background values or high variations may indicate washing problems.

3. All washing must be performed with the provided washing buffer diluted to usage concentration. Washing volume per washing cycle and well must be 300 µL at least.
4. The danger of handling with potentially infectious material must be taken into account.
5. When using an automatic microtiter plate washer, the respective instructions fur use must be carefully followed. Device adjustments, e.g. for plate geometry and the provided washing parameters, must be performed. Dispensing and aspirating manifold must not scratch the inside well surface. Provisions must be made that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, this could be controlled, and possible remaining fluid could then be removed, by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.
6. Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a multistepper device, a multichannel pipette, or a squirt bottle. The fluid may be removed by dynamically swinging out the microtiter plate over a basin. If aspirating devices are used, care has to be taken that the inside well surface is not scratched. Subsequent to every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on non-fuzzy absorbent tissue.

Assay Step

1. In each test, a negative and a positive control has to be used. The positive control must also be tested under neutralising conditions. To confirm positive results this is also advisable for samples. Generally, double determinations are recommended.

   - All wells needed are filled with either 50 µL Dilution Buffer (D) or 50 µL neutralising dilution buffer (1:10 diluted neutralising serum C) each.

   - As negative control add 50 µL Dilution Buffer (D), as positive control 50 µL HAV antigen (B) are added. Stool and other specimen are also added 50 µL/well (double determination is recommended). Ultimately, each well has to be filled with 100 µL liquid.
2. Seal the plate with adhesive tape and incubate it for 2 hours at 37°C.
3. At the end of the incubation period the wells are evacuated (attention: infective agent) and washed 3 times with 300 µL Wash Buffer (G) per well with 10 seconds incubation time respectively.and empty the wells.
4. Add 100 µL diluted Conjugate Solution A per well, reseal the plate and incubate for another 2 hours at 37°C.
5. At the end of the incubation period the wells are evacuated and washed 3 times with 300 µL Wash Buffer (G) per well with 10 seconds incubation time respectively and empty the wells.
6. Add 100 µL Substrate (E) per well and incubate for 30 minutes in the dark.
7. After the incubation 100 µL Stop Solution (F) is added into each well. The colour of positve reactions will turn from blue to yellow.
8. The measurement of the colour is performed at 450 nm. The reference wavelength in dual wavelength mode should be ≥ 590 nm.

Calculation

positive control value 1: 1.114

positive control value 2: 1.162

Average: (1,114 + 1,162) : 2 = 1.138

negative control value 1: 0.024

negative control value 2: 0.030

Average: (0,024 + 0,030) : 2 = 0.027

Subtract the blank (negative control):

Positive control: 1.138 – 0.027 = 1.111

10 % extinction positive control (cut-off): 1.111 x 0.10 = 0.111

15 % extinction positive control: 1.111 x 0.15 = 0.166

Samples with extinction higher than 0.111 are regarded as positive if the value of the positive control on neutralising conditions declines more than 80 % and the sample value itself declines more than 25 % respectively.

Samples with extinctions between the cut-off value of 0.111 and 0.166 are recommended to be measured again - if a reduction of 25 % is achieved under neutralising conditions.

Interpretation Of Results

1. Calculate the average of the multiple values. Subtract the negative control value (blank) from all measured values (could be done automatically by many readers as blank correction). The difference between the positive and negative control must be at least 0,5 OD - otherwise the test is considered invalid.
2. The drop of the positive control value caused by neutralising serum must be more than 80 %.
3. The cut-off value is 10 % of the positive control value. Samples with extinction equal or slightly higher than the cut off value (extinctions between 10 to 15 % of the positive control) are recommended to be analysed again. In case of a comparable result, the sample is regarded as positive - similar to samples with average values higher than 15 % of the positive control.
4. However, this is only true if the extinction of the positive samples declines at least 25 % under neutralising conditions (incubation of the samples with neutralising buffer). Otherwise, the result cannot be regarded as positive.
5. Positive samples with values higher than the Positive Control (B), which do not decline more than 25 % by neutralisation, must be diluted 1:10 in Dilution Buffer (D) and tested again. Sample values with a negative value after subtraction of the blank can appear, nevertheless such test is valid.

Precision

Cut-off: 16 % CV intra-assay

Positive control: 3.5 % CV intra-assay

Sensitivity

91 % of the Elisa-positive samples were also HAV-PCR positive.

Precautions

1. The CD HAV Antigen ELISA Kit is for in-vitro use only.
2. The antigen of the positive control has been inactivated with formaldehyde. Reagents of human origin have been tested for HBsAg and antibodies to HIV and HCV and been found to be negative. Nevertheless, such tests are unable to prove the complete absence of infectious agents. Therefore, all reagents should be handled with appropriate precautions.
3. Do not pipette by mouth. Wear disposable gloves throughout the test procedure. In case of spills, bench-tops and instruments must be disinfected.
4. Disposable materials should be treated as infectious waste.
5. The stop solution contains sulfuric acid and is therefore corrosive. On contact wash immediately with running water- if necessary, contact a doctor.
6. Acidic waste should be neutralised before disposal.