HAV-Antigen EIA

Enzyme Immuno Assay for the Detection of Hepatitis A-Virus Antigen

Product Code E12

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For In-Vitro-Use only

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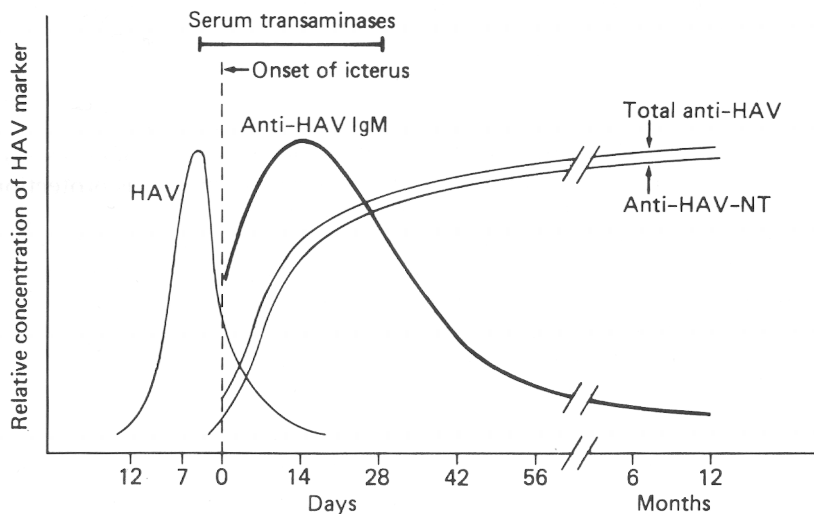
INTENDED USE:

The **mediagnost** HAV-ANTIGEN EIA E12 is an enzyme immunoassay for the identification of Hepatitis A virus in stool and cellculture.

INTRODUCTION:

A positive identification of Hepatitis A Virus (HAV) in the stool of patients 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, patients two weeks after onset of icterus.



The detection of HAV in specimen other than stool is also possible with the mediagnost HAV-ANTIGEN EIA E12, for example in lysates of HAV infected cells or in culture supernatants. If necessary 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).

TEST PRINCIPLE

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.

A positive reaction must be confirmed by neutralising with anti-HAV serum to discriminate false positive reactions which sometimes occure in stool.

KIT CONTENTS

1) **Test plate:**

Test plate with 96 wells, divided into 12 removable strips with 8 wells each, coated with monoclonal antibody against HAV antigen.

2**) Conjugate concentrate (flask A):**
1 vial (250 µl) conjugate, (mouse monoclonal anti HAV IgG, peroxidase conjugated) 100 x concentrated.

3) Positive controle (flask B):

1 vial (1 ml) positive controle. Hepatitis A-Virus antigen, inactivated, ready for use.

4) **Neutralising serum (flask C):**
1 vial (1 ml) anti-HAV-positive serum, 10 x concentrated.

5) **Dilution buffer (flask D):**
1 vial (120 ml) Dilution buffer for specimen und conjugate, red coloured, ready for use.

6) **Substrate (flask E):**

1 vial (13,5 ml), ready for use.

7) **Stop solution (flask F):**
1 vial (13,5 ml) stop solution, 0,2 M sulphuric acid, ready to use.
Caution: Acid!

8) **Wash buffer (flask G):**

1 vial (60 ml) wash buffer, 20 x concentrated.

STORAGE

All materials must be stored at 2-8 °C in the dark. Strips not used immediately should be stored airseal in the laminate bag together with desiccant. The shelf-life of the components after opening is not affected, if used appropriately.

MATERIALS NOT PROVIDED

- Distilled water for dilution of wash buffer
- Centrifuge for preparation of stool specimen.
- Adhesive tape to seal the reaction chambers.
- Incubator or water bath with an adaptor for microtiter plates.
- Precision pipettes with disposable tips.
- Washing apparatus for microtiter plates.
- Photometer for microtiter plates ("ELISA-Reader").

PRECAUTIONS

1) The **mediagnost** HAV-ANTIGEN EIA E12 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 sulphuric 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.

7) Reagents contain as preservative Thimerosal, however, highly diluted (0.01%). Thimerosal is very toxic when swallowed and it involves a certain danger of cumulative effects (R-Phrases 26/27/28-33-50/53 and S 13-28.1-36-45-60-61).

First aid procedures:

Scin contact: Wash affected area thoroughly with water. Discard contaminated cloths and shoes.

Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to assure an effectual rinsing spread the eyelids.

Ingestion: If swallowed, wash out mouth throughly with water. Immediately see a physician.The Stop Solution provided is an acid solution. Avoid direct contact. Wear eye, hand, face and clothing protection when using this material.

TEST PREPARATION:

1) Bring all reagents to room temperature before use

2) Wash buffer:(G) : Dilute the 20 x wash buffer 1:20 with distilled water.

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.

SAMPLE PREPARATION

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

Supernatants of cellculture and cellysates can be used directly. If required they can be concentrated i.e. with ultracentrifugation.

TEST PROTOCOL

1) In every test, two negative and two positive controls should be performed, and also two positivecontrols under neutralising conditions.

All wells needed are filled with eather 50 µl dilution buffer (D) or 50 µl neutralising dilution buffer (1:10 diluted neutralising serum) each.

Add dilution buffer (D) as negative control and the positive control (B), HAV antigen, 50 µl/well respectively to the preincubated wells. Stool and other specimen are also added 50 µl/well (double determination is recommended). Each well is 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 5 times with 300 µl wash buffer per well with 10 seconds incubation time respectively.and empty the wells.

4) Add 100 µl diluted conjugate solution 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 5 times with 300 µl wash buffer per well with 10 seconds incubation time respectively and empty the wells.

6) Add 100µl substrate (E) per well and incubate for 30 minutes in the dark.

7) After the incubation 100 µl stop solution 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 wave length in dual wave length mode should be between 570 and 650 nm.

RESULTS

Calculate the average of the multible 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 otherwise the test is considered invalid.

The drop of the positive control value caused by neutralising serum must be more than 80 %.

The cut-off calculation is 10 % of the positive control value.

POSITIVE SAMPLES

Samples with extinction equal or higher than the cut off value are regarded as positive. The extinction of the positive samples should decline under neutralizing condition at least 25 % otherwise the result is regarded not as positive. Positive samples with values higher than the positive control which don't decline more than 25 % by neutralisation must be diluted 1:10 in dilution buffer (D) and tested again. Sample values with a negative sign after subtraction of the blank could be found, nevertheless such test is valid.

CALCULATION (EXAMPLE)

positive controle value 1 : 1,114
positive controle value 2 : 1,162
Average : (1,114 + 1,162) : 2 = 1,138

negative controle value 1 : 0,024
negative controle value 2 : 0,030
Average : (0,024 + 0,030) : 2 = 0,027

Subtract the blank (negative control):

Positive controle: 1,138 - 0,027 = 1,111

Cut-off : 1,111 / 10 = 0,111

Samples with extinction higher than 0,111 are regarded as positive if the value of the positive control on neutralizing conditions declines more than 80 % and the sample value itself declines more than 25 %

LIMITATIONS:

Test components are for in-vitro-use only.

Sensitivity:

Elisa-negative samples nevertheless can contain HAV particles.

Specificity:

91% of the elisa-positive samples were also HAV-PCR positive.¨ Cross reactivity is not known.

Precision:

cut-off: 16%CV intra-assay
positive control: 3,5%CV intra-assay