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User's Manual

Hepatitis A Virus Antigen ELISA

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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 Hepatitis A Virus Antigen ELISA is for the semi-quantitative determination of Hepatitis A Virus (HAV) antigen in human stool and cell culture supernatant. For Research Use Only. Not for use in diagnostic procedures.

Principles of Testing

Samples are pipetted into wells of a microtiter plate coated with antibodies directed against Hepatitis A Virus (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 with 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 addition of stop solution. The blue color of a positive reaction turns to yellow and is measured in a microplate reader at 450 nm. The intensity of the color indicates the concentration of bound HAV antigen. A positive reaction must be confirmed by re-testing with anti-HAV neutralizing serum to discriminate false positive reactions which sometimes occur in stool.

Reagents And Materials Provided

1. Microtiter Plate: Microtiter plate with 96 wells, divided into 12 separable strips with 8 wells each, coated with monoclonal antibody against HAV antigen.
2. Conjugate Concentrate (A): 1 vial (150 µL) conjugate, (mouse monoclonal anti-HAV IgG, peroxidase conjugated) 100× concentrated.
3. Positive Control (B): 1 vial (500 µL) positive control. Hepatitis A-Virus antigen, inactivated, ready for use.
4. Neutralizing Serum (C): 1 vial (500 µL) anti-HAV-positive serum, 10X concentrated.
5. Dilution Buffer (D): 1 vial (120 mL) Dilution buffer for sample and conjugate, red colored, ready for use.
6. Substrate (E): 1 vial (12 mL), ready for use.
7. Stop Solution (F): 1 vial (12 mL) stop solution, 0.2 M sulfuric acid, ready for use. Caution: Acid!
8. Wash Buffer (G): 1 vial (50 mL) wash buffer, 20× concentrated.
9. Sealing Tape: For covering of the microtiter plate, 2×, adhesive.

Materials Required But Not Supplied

1. Incubator or water bath with an adapter for microtiter plates (37 °C)
2. Centrifuge for preparation of stool samples
3. Precision pipettes and multichannel pipettes with disposable plastic tips
4. Distilled or deionized water for dilution of the Wash Buffer (WB)
5. Microtiter plate washer (recommended)
6. Microtiter plate reader ("ELISA Reader") with filter for 450 and ≥ 590 nm

7. Polyethylene (PE)/Polypropylene (PP) tubes for dilution of samples
8. Timer

Storage

All materials must be stored at 2-8°C in the dark. Unused microtiter plate strips must be sealed with desiccant at 2-8°C. The shelf-life of the components after opening is not affected, if used appropriately.

Specimen Collection And 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 cell culture and cell lysates can be used directly. If required, they can be concentrated i.e. with ultracentrifugation.

Reagent Preparation

Bring all reagents to room temperature (20-25°C) before use

1. Wash Buffer (G): Dilute the 20× Wash Buffer 1:20 with distilled water. After dilution the 1× Wash Buffer is only stable for 4 weeks, please dilute only the amount required.

2. Dilute neutralizing serum (C): 1:10 with dilution buffer (D), for confirmation of positive reactions.

Dilute only the volume used in the test (50 µl per well). Diluted serum is stable for at least one week at 4°C.

3. Dilute the 100× 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

Incubation at room temperature means 20-25°C

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 variations of measured optical densities, potentially leading to false results and erroneous sample values. Effects like high background values or high variations may indicate washing problems. All washing must be performed with the provided washing buffer diluted to working concentration. Washing volume per washing cycle and well must be at least 300 µl. The danger of handling potentially infectious material must be considered.

Automated Washing: When using an automatic microtiter plate washer, the respective instructions for 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. Ensure that the remaining fluid volume of every aspiration step is minimized. Following the last aspiration step of each washing cycle, tap the plate and dry on lintfree absorbent tissue (i.e., paper towel).

Manual Washing: Manual washing is an adequate alternative option. Washing Buffer may be dispensed via a repeat pipette, 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 must be taken that the inside well surface is not scratched. After every single washing step, the remaining fluid should be removed by inverting

the plate and repeatedly tapping it dry on lint-free absorbent tissue (i.e., paper towel).

Test Protocol

***Note:**

Two criteria are required to evaluate a sample as positive.

1) The OD must be equal to or higher than the cut-off value.
2) The OD of the sample must be decline by at least 25% under neutralizing conditions.

Thus, two tests must be run to confirm a positive sample.

Users may simultaneously run samples with dilution buffer and neutralizing dilution buffer in one run or carry out a complete run in dilution buffer; and a subsequent run of positive sample with neutralizing serum.

1. In every test, two negative and two positive controls should be tested, along with two positive controls under neutralizing conditions. All wells needed are filled with either 50 µl Dilution Buffer (D) or 50 µl neutralizing dilution buffer (1:10 diluted neutralizing serum). Add 50 µL/well of Dilution Buffer (D) to the negative control wells and HAV antigen to the positive control (B) wells. Stool and other samples are also added 50 µl/well to the appropriate wells. Duplicate determination is recommended. Each well is filled with 100 µl of liquid in total.
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 emptied (attention: infective agent) and washed
3 times with 300 µl Wash Buffer (G) per well with 10 seconds incubation time per wash. After every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue (i.e., paper towel).
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 emptied (attention: infective agent) and washed 3 times with 300 µl Wash Buffer (G) per well with 10 seconds incubation time per wash. After every single washing step, the remaining fluid should be removed by inverting the plate and repeatedly tapping it dry on lint-free absorbent tissue (i.e., paper towel).
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 color of positive reactions will turn from blue to yellow.
8. The measurement of the color is performed at 450 nm. The reference wavelength in dual wavelength mode should be ≥ 590 nm.

Calculation

Calculate the average of the replicates. 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 neutralizing serum must be more than 80%. The cut-off calculation is 10% of the positive control value.

Samples with extinction equal or higher than the cut off value are regarded as positive. The extinction of the positive samples should decline at least 25% under neutralizing condition otherwise the result is regarded as not positive. Positive samples with values higher than the Positive Control (B) which do not decline more than

25% by neutralization 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 a result is valid.

Calculation example:

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

Cut-off: 1.111 / 10 = 0.111

In the example, 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%.

Precision

Cut-off: 16% CV intra-assay

Positive control: 3.5% CV intra-assay

Specificity

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

Precautions

This kit is for research use only, not for internal applications in humans or animals. This product must be used as described in the enclosed package insert provided with the kit.

1. Do not use obviously damaged, microbially contaminated, or spilled material.
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. Disposable materials should be treated as infectious waste Therefore, all reagents should be handled with appropriate precautions.
3. Appropriate precautions and good laboratory practices must be used in the storage, handling, and disposal of the kit reagents. The disposal of the kit components must be made according to the local regulations.
4. Material Safety Data Sheet is available upon request.
5. Do not pipette by mouth. Wear disposable gloves throughout the test procedure. In case of spills, bench-tops and instruments must be disinfected.
6. The stop solution contains sulfuric acid and is therefore corrosive. On contact wash immediately with

running water- if necessary, contact a doctor.

7. Acidic waste should be neutralized before disposal.

General First Aid Procedures

1. Skin contact: Wash affected area thoroughly with water. Discard contaminated clothes and shoes.
2. Eye contact: In case of contact with eyes, rinse immediately with plenty of water at least 15 minutes. In order to ensure effective rinsing, hold the eyelids open.
3. Ingestion: If swallowed, wash out mouth thoroughly 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.

Limitations

ELISA-negative samples nevertheless can contain HAV particles.