HAV-Antigen ELISA

Enzyme Immunoassay for the Detection of

Hepatitis A-Virus Antigen

For Research Use Only.
Not for use in diagnostic procedures.


REF **E12**

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Symbols/ Symbole /Symboles/ Simboli/ Símbolos/ Símbolos/ Symbolen/ Symboler/ Symboler/ Symbole/ Szimbólumok/ Symboly/ Symboly/ Символи/ Sümbolid/ Σύμβολα/ Simboluri/ Simboli/ Symbolit

according to DIN EN 980 and EDMA recommendations Standard News 6 2001

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	Store at between/ Lagerung bei zwischen/ Conserver à entre/ Conservare a tra/ Conservar a temp. Entre/ Armazenar entre/ Bewaar bij tussen/ Opbevares mellem/ Förvaras vid/ Przechowywać w/ Tárolási tartomány/ Skladujte v rozsahu / Skladujte v rozmezí/ Температурно ограничение/ Säilitada temperatuuridel/ Φύλαξη σε θερμοκρασία/ Depozitare între/ Skladiščenje med/ Säilytys x-y Celsiusasteen lämpötilassa
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	Keep away from sunlight/ Nicht dem Sonnenlicht aussetzen/ Conserver à l´abri de la lumière/ Conservare al riparo della luce solare/ No exponer a la luz solar/ Proteger da luz solar/ Niet aan zonlicht blootstellen/ Må ikke udsættes for sollys/ Utsätt inte för solljus/ Nie wystawiać na słońce/ Napfénytől távol tartandó/ Nevystavovať slnečnému svetlu/ Nevystavovat slunečnímu světlu/ Да се предпазва от слънчева светлина/ Kaitsta otsese päikesekiirguse eest/ Κρατήστε το μακριά από την ηλιακή ακτινοβολία/ Ţineţi departe de lumina soarelui/ Ne izpostavljajte sončni svetlobi/ suoja auringonvalolta

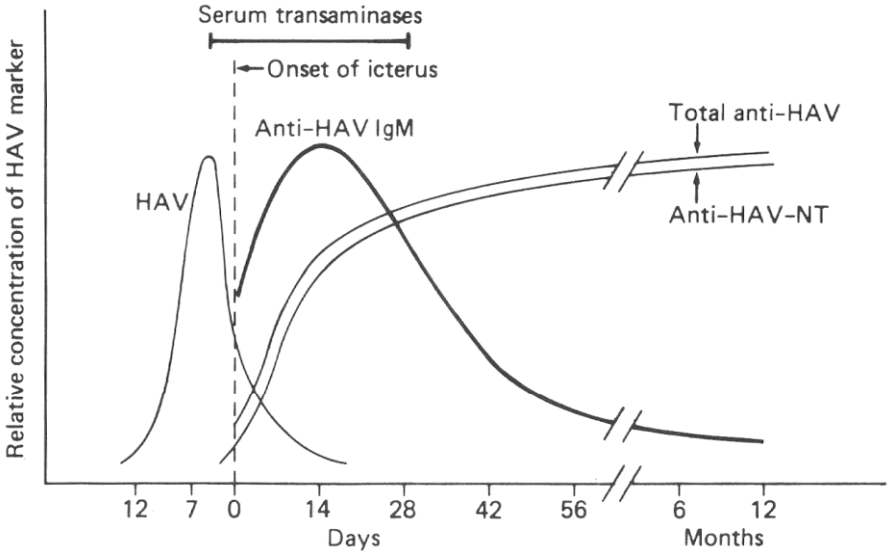
For Research Use Only.
Not for use in diagnostic procedures.
CAUTION: Not for human or animal therapeutic or diagnostic use.
For in vitro use only!
For professional use only!
Read entire protocol before use!

INTENDED USE

The **mediagnost** HAV-ANTIGEN ELISA E12 is an enzyme immunoassay **for research use** for the identification of Hepatitis A virus in stool and cell culture.

INTRODUCTION

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 mediagnost HAV-ANTIGEN ELISA E12, 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).

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.

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.

KIT CONTENTS

1) **Microtiter Plate:**
   Microtiter 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 (150 µL) conjugate, (mouse monoclonal anti HAV IgG, peroxidase conjugated) 100 x concentrated.

3) **Positive Control (flask B):**
   1 vial (500 µL) positive control. Hepatitis A-Virus antigen, inactivated, ready for use.

4) **Neutralising serum (flask C):**
   1 vial (500 µL) 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 (12 mL), ready for use.

7) **Stop solution (flask F):**
   1 vial (12 mL) stop solution, 0.2 M sulphuric acid, ready to use.
   Caution: Acid!

8) **Wash buffer (flask G):**
   1 vial (50 mL) wash buffer, 20 x concentrated.

9) **Sealing tape**
   for covering of the microtiter plate, 2 x, adhesive

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.

MATERIALS NOT PROVIDED

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

PRECAUTIONS

1) The mediagnost HAV-ANTIGEN ELISA 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) The Reagents **A, B, C, D** contain as preservative (0.01%) 2-Methyl-4-isothiazolin-3-one Solution

H317	May cause an allergic skin reaction
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention
P302+P352	IF ON SKIN: Wash with plenty of soap and water
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

8) The Reagents **A, C, D, G** contain as preservative (0,01%) (w/w) 5-chloro-2-methyl 2H isothiazol-3-one and 2-methyl-2H-isothiazol-3-one

H317	May cause an allergic skin reaction
P280	Wear protective gloves/protective clothing/eye protection/face protection.
P272	Contaminated work clothing should not be allowed out of the workplace.
P261	Avoid breathing dust/fume/gas/mist/vapours/spray.
P333+P313	If skin irritation or rash occurs: Get medical advice/attention
P305+P351	IF IN EYES: Rinse cautiously with water for several minutes.
P338	Remove contact lenses, if present and easy to do. Continue rinsing.
P302+P352	IF ON SKIN: Wash with plenty of soap and water
P501	Dispose of contents/container in accordance with local/regional/national/international regulations.

First aid procedures:

Skin 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 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.

TECNICAL NOTES

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 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.

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.

The danger of handling with potentially infectious material must be taken into account.

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.

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.
Incubation at room temperature means: 20-25°C

TEST 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.

SAMPLE 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.

TEST PROTOCOL

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.**

RESULTS

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.

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

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.

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.

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.

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
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.

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

Summary of the Assay

Reagent preparation:	**Dilution:**
Conjugate Concentrate A	**1:100** with **Dilution Buffer D;** Dilute only the volume used in the test
Neutralising serum C	**1:10** with **Dilution Buffer D;** Dilute only the volume used in the test
Washing Buffer G	**1:20** with **Aqua dest.**
Stool samples	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.

Assay Procedure in Double Determination

Pipette	**Reagents**	**Position**
50 µL	Pipette Dilution Buffer **D** or Neutralising Dilution Buffer (**1:10** Dilution of the Neutralising Serum **C**, see above) in each well a) analyse all samples including positive control with **both** buffers **or** b) analyse only positive control with **both** buffers; test initially positive samples in a 2nd assay under neutralising conditions (see recommendation)	In **all** wells required
50 µL	Add Dilution Buffer **D** (Negative-Control) in positions:	**A1/A2**
50 µL	Add **Samples** and positive control (HAV-Antigen **B**) in	following wells
Cover the wells with the sealing tape.		
Incubation: 2 h at **37°C**		
3x 300 µL	Aspirate the contents of the wells and wash 3x with **300 µL** Wash Buffer **G**.	each well
100 µL	Diluted **Conjugate Solution A**	each well
Cover the wells with the sealing tape.		
Incubation: 2 h at **37°C**		
3x 300 µL	Aspirate the contents of the wells and wash 3x with **300 µL** Wash Buffer **G**.	each well
100 µL	Substrate Solution **E**	each well
Incubation: 30 min in the Dark at RT		
100 µL	Stopping solution **F**	each well
Measure the absorbance within **30 min** at **450 nm** (Reference wavelength **≥ 590 nm)**		