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Anti-HAV IgM

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REF	Σ	SYSTEM
11820591 122	100	MODULAR ANALYTICS E170 **cobas e** 411 **cobas e** 601 **cobas e** 602

English

Intended use

Immunoassay for the in vitro qualitative determination of IgM antibodies to the hepatitis A virus in human serum and plasma. The assay is used as an aid to detect an acute or recently acquired hepatitis A virus infection.

The **e**lectro**c**hemi**l**uminescence **i**mmuno**a**ssay "ECLIA" is intended for use on Elecsys and **cobas e** immunoassay analyzers.

System information

For **cobas e** 411 analyzer: test number 480
For MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 085

Summary

The hepatitis A virus is a RNA-containing virus that lacks an envelope. It belongs to the family of picornaviruses. To date, just one human serotype and 7 genotypes have been described. The viral capsid consists of 3 proteins (VP1-VP3) that form an immunodominant structure on the surface of the viral particle that is highly conserved between all genotypes. After vaccination or natural infection, the immune response is directed against this structure.[1]

Hepatitis A is the most common form of acute viral hepatitis. It is transmitted by the fecal-oral route. The disease has not been known to take a chronic course, nor does the virus persist in the organism.[2]

An acute hepatitis A infection can be assumed if anti-HAV IgM antibodies are detected. Anti-HAV IgM antibodies can always be detected at the onset of the disease, and usually disappear 3 to 4 months later.[3,4,5] Anti-HAV IgM can also be detected in some patients for a longer period of time, however.[6] HAV IgM antibodies develop only very rarely after vaccination.[7,8]

Assays to detect anti-HAV IgM antibodies are used in the differential diagnosis of acute hepatitis to determine a hepatitis A infection.

Test principle

µ-Capture test principle. Total duration of assay: 18 minutes.

- 1st incubation: Pretreatment of 10 µL of the automatically 1:400 diluted sample (using Diluent Universal) with anti-Fdγ reagent to block specific IgG in the presence of monoclonal anti-HAV antibodies labeled with ruthenium complex[a)].
- 2nd incubation: After addition of biotinylated monoclonal h-IgM-specific antibodies, HAV antigen, and streptavidin-coated microparticles, the anti-HAV IgM antibodies present in the sample form a sandwich complex with the HAV antigen and the ruthenium-labeled anti-HAV antibody which becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as A-HAVIGM.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HAV Ab~$Ru(bpy)_3^{2+}$ (gray cap), 1 bottle, 10 mL:
Monoclonal Anti-HAV antibody (mouse) labeled with ruthenium complex 0.15 µg/mL; anti-human-Fdγ antibody (sheep) 0.04 mg/mL; HEPES[b)] buffer 50 mmol/L, pH 7.2; preservative.

R2 Anti-h-IgM Ab~biotin; HAV Ag (black cap), 1 bottle, 10 mL:
Biotinylated monoclonal anti-h-IgM antibody (mouse) 0.4 µg/mL; HAV antigen (cell culture), 25 U/mL (Roche units); HEPES buffer 50 mmol/L, pH 7.2; preservative.

b) HEPES = [4-(2-hydroxyethyl)-piperazine]-ethane sulfonic acid

A-HAVIGM Cal1 Negative calibrator 1 (white cap), 2 bottles of 0.67 mL each:
Human serum, negative for anti-HAV IgM; preservative.

A-HAVIGM Cal2 Positive calibrator 2 (black cap), 2 bottles of 0.67 mL each:
Anti-HAV IgM (human) approximately 5 U/mL (Roche units) in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

All human material should be considered potentially infectious.

The calibrators (A-HAVIGM Cal1 and A-HAVIGM Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV.

The testing methods used assays approved by the FDA or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HAV IgM and the HAV antigen (cell culture) were inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[9,10]

Avoid foam formation in all reagents and sample types (specimens, calibrators and controls).

Reagent handling

The reagents in the kit are ready-for-use and are supplied in bottles compatible with the system.

cobas e 411 analyzer: The calibrators should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the ready-for-use calibrators into empty snap-cap bottles (CalSet Vials). Attach the supplied labels to these additional bottles. Store the aliquots at 2-8 °C for later use.

Perform **only one** calibration procedure per aliquot.

All information required for correct operation is read in from the respective reagent barcodes.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn

the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the Elecsys reagent kit **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	8 weeks

Stability of the calibrators	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 at 20-25 °C	use only once

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

Serum collected using standard sampling tubes or tubes containing separating gel.

Li-, Na-heparin, K_3-EDTA and sodium citrate plasma.

Criterion: Recovery within 90-110 % of the serum value.

Stable for 7 days at 2-8 °C, 6 months at -20 °C. The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates and thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples, calibrators and controls on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 4 bottle labels

Materials required (but not provided)

- REF 11876368122, PreciControl Anti-HAV IgM, for 16 x 0.67 mL
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 1776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- MODULAR ANALYTICS E170 or **cobas e** analyzer

Accessories for **cobas e** 411 analyzer:

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, AssayCup, 60 x 60 reaction cups
- REF 11706799001, AssayTip, 30 x 120 pipette tips
- REF 11800507001, Clean-Liner

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup, 48 magazines x 84 reaction cups or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, ISE Cleaning Solution/Elecsys SysClean, 5 x 100 mL system cleaning solution

Assay

For optimum performance of the assay follow the directions given in this document for the analyzer concerned. Refer to the appropriate operator's manual for analyzer-specific assay instructions.

Resuspension of the microparticles takes place automatically prior to use. Read in the test-specific parameters via the reagent barcode. If in exceptional cases the barcode cannot be read, enter the 15-digit sequence of numbers (except for the **cobas e** 602 analyzer).

Bring the cooled reagents to approximately 20 °C and place on the reagent disk (20 °C) of the analyzer. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators in the sample zone.

All the information necessary for calibrating the assay is automatically read into the analyzer.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against a Roche reference standard. The units have been selected randomly.

Calibration frequency: Calibration must be performed once per reagent lot using A-HAVIGM Cal1, A-HAVIGM Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Calibration interval may be extended based on acceptable verification of calibration by the laboratory.

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings with PreciControl Anti-HAV IgM outside the defined limits
- more frequently when this is required by pertinent regulations

Quality control

For quality control, use PreciControl Anti-HAV IgM.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

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Note:
For technical reasons re-assigned target values valid only for a specific reagent and control lot combination must be entered manually on all analyzers (except for the **cobas e** 602 analyzer). Therefore always refer to the value sheet included in the reagent kit or PreciControl kit to make sure that the correct target values are used.
When a new reagent or control lot is used, the analyzer will use the original values encoded in the control barcodes.

Follow the applicable government regulations and local guidelines for quality control.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of A-HAVIGM Cal1 and A-HAVIGM Cal2.

The result of a sample is given either as reactive or non-reactive as well as in the form of a cutoff index (signal sample/cutoff).

Interpretation of the results

Samples with a cutoff index ≥ 1.0 are reactive in the Elecsys Anti-HAV IgM assay. These samples are considered positive for anti-HAV IgM.

Samples with a cutoff index < 1.0 are non-reactive in the Elecsys Anti-HAV IgM assay. These samples are considered negative.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 855 µmol/L or < 50 mg/dL), hemolysis (Hb < 1.09 mmol/L or < 1.75 g/dL), lipemia (Intralipid < 2000 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Correct assignment of negative and positive samples.

Samples should not be taken from patients receiving therapy with high biotin doses (i.e. > 5 mg/day) until at least 8 hours following the last biotin administration.

No interference was observed from rheumatoid factors up to a concentration of 3200 IU/mL.

The high-dose hook effect does not lead to false-negative results in the Elecsys Anti-HAV IgM assay.

In vitro tests were performed on 18 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to high titers of antibodies to immunological components, streptavidin or ruthenium can occur.

These effects are minimized by suitable test design.

As with many µ-capture assays, an interference with unspecific IgM is observed. Increasing amounts of unspecific IgM may lead to a decrease in the recovery of positive samples with the Elecsys Anti-HAV IgM assay.

For diagnostic purposes, the results should always be assessed in conjunction with the patient's medical history, clinical examination and other findings.

Dilution

Use Diluent Universal for automatic sample predilution. This also applies if an additional sample dilution is necessary.

Expected values

The cutoff is selected such that the anti-HAV IgM concentration is above the cutoff index when acute HAV infection is present. In case of a past hepatitis A infection, the anti-HAV IgM concentration is usually below the cutoff index of 1.0.

In the course of most acute hepatitis A infections, the anti-HAV IgM concentration decreases within 3-4 months after onset of the first symptoms and can then no longer be detected. Anti-HAV IgM antibodies are persistent only in exceptions and can then be detected beyond this period.[3,4,5]

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human sera and controls (repeatability n = 21, intermediate precision n = 10); intermediate precision on MODULAR ANALYTICS E170 analyzer was determined in a modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60). The following results were obtained:

cobas e 411 analyzer						
	Repeatability			**Intermediate precision**		
Sample	Mean COI[c)]	SD COI	CV %	Mean COI	SD COI	CV %
HS[d)], negative	0.28	0.006	2.0	0.21	0.008	3.8
HS, weakly positive	1.10	0.037	3.4	1.05	0.029	2.8
HS, positive	11.7	0.361	3.1	11.8	0.643	5.4
PC[e)] A-HAVIGM1	0.25	0.005	2.0	0.22	0.006	2.8
PC A-HAVIGM2	2.30	0.106	4.6	2.21	0.059	2.7

c) COI = cutoff index

d) HS = human serum

e) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean COI	SD COI	CV %	Mean COI	SD COI	CV %
Human serum 1	0.31	0.004	1.3	0.31	0.008	2.5
Human serum 2	0.96	0.020	2.1	0.97	0.049	5.0
Human serum 3	2.54	0.059	2.3	2.55	0.141	5.5
PC A-HAVIGM1	0.28	0.006	2.0	0.29	0.008	2.6
PC A-HAVIGM2	1.70	0.071	4.2	1.94	0.154	7.9

Analytical specificity

No cross-reactions with anti-HAV IgG, HBV, HCV, CMV, EBV, HSV, Rubella, and Toxoplasma gondii were observed.

Measurements were performed on each of the pathogens listed above using ≥ 9 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (ANA, AMA).

Clinical sensitivity

Individual samples of patients during an acute phase of the HAV infection:

In 211/211 individual samples of clinically characterized patients with an acute HAV infection, anti-HAV IgM antibodies were detected with the Elecsys Anti-HAV IgM assay and an anti-HAV IgM comparison test. The 95 % confidence range for the sensitivity is 98.3-100 %.

Samples of monitored patients after an acute HAV infection:

Anti-HAV IgM was measured in a total of 147 samples from 45 monitored patients after an acute HAV infection using the Elecsys Anti-HAV IgM assay and an anti-HAV IgM comparison test.

122 samples were consistently positive, 14 samples were consistently negative. 10 out of 11 discrepant samples were from patients in the recovering phase (> 4 months after the first symptoms showed). 9 of these samples were negative with the Elecsys Anti-HAV IgM assay while they were positive or showed borderline values with the comparison test.

One sample which was weakly positive with the Elecsys Anti-HAV IgM assay showed a borderline result with the comparison test.

One sample which was positive with the Elecsys Anti-HAV IgM assay was negative with the comparison test. This sample from a very early HAV seroconversion phase was confirmed positive with a third anti-HAV IgM test.

Clinical specificity

Samples from blood donors which had not been selected were used to determine the specificity. All 1032 samples of these donors were negative with the Elecsys Anti-HAV IgM assay.

280/280 samples from hospitalized patients, pregnant women, dialysis patients and drug addicts with no indication of an HAV infection were negative with both the Elecsys Anti-HAV IgM assay and the comparison test.

One additional sample of a pregnant woman was weakly positive with both tests. The specificity in both studies is 100 %. The 95 % confidence range is 99.7-100 %.

Anti-HAV IgM

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References

1 Robertson BH, Nainan OV. Genetic and antigenetic variants of hepatitis A virus. In: Viral Hepatitis and Liver Disease. Eds: Rizzeto M, Purcell RH, Gerin JL, Verme G, Edizioni Minerva Medica, Turin 1997;14-18.

2 Koff RS. Hepatitis A. Lancet 1998;341:1643-1649.

3 Stapleton JT. Host Immune Response to Hepatitis A Virus. JID 1995;171(Suppl 1):9-14.

4 Gust I. Diagnosis. In: Viral Hepatitis. Eds Zuckerman AJ, Thomas HC, Churchill Livingstone, 1995;55-59.

5 Bower WA, Nainan OV, Han X, et al. Duration of Viremia in Hepatitis A Virus Infection. JID 2000;182:12-17.

6 Lemon ML, Days SL. Type A hepatitis. In: Gorbach S, Bartlett JG, Blacklown NL (eds). Infectious Diseases. Saunders WB, Philadelphia, 1992;705-708.

7 Sjogren MH, Hoke CH, Binn LN, et al. Immunogenicity of an Inactivated Hepatitis A Vaccine. Ann Intern Med 1991;114:470-471.

8 Shouval D, Ashur Y, Adler R, et al. Single and booster dosae response to an inactivated hepatitis A virus vaccine: comparison with immune serum globulin prophylaxis. Vaccine 1993;11,Suppl 1:9-14.

9 Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.

10 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information and the Method Sheets of all necessary components (if available in your country).

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see https://usdiagnostics.roche.com for definition of symbols used):

CONTENT	Contents of kit
SYSTEM	Analyzers/Instruments on which reagents can be used
REAGENT	Reagent
CALIBRATOR	Calibrator
→	Volume after reconstitution or mixing
GTIN	Global Trade Item Number

CE

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