Elecsys Anti-HAV IgM

cobas®

REF		Σ	SYSTEM
07026773190	07026773500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
AHAVIGM	10162

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 **cobas e** immunoassay analyzers.

Summary

The hepatitis A virus (HAV) is a non-enveloped single stranded RNA-virus that belongs to the family of picornaviruses. To date, just one human serotype and 6 genotypes have been described, 3 of which infect humans (genotypes I, II and III).[1] Initially, 7 genotypes were described, but subsequent analyses suggest that genotypes II and VII are subtypes of genotype II.[2] The viral capsid consists of 3 major structural proteins (VP1-VP3) and a fourth putative protein (VP4) that form an immunodominant structure on the surface of the viral particle, which is highly conserved between all genotypes. After vaccination or natural infection, the immune response is directed against this structure.[1,3]

HAV is one of the most common causes of infectious jaundice and is transmitted by the fecal-oral route. HAV causes acute hepatitis and is not associated with chronic liver disease, nor does the virus persist in the organism.[1,3] Serologic testing for detection of immunoglobulin M (IgM) antibodies to HAV is required for differential diagnosis of acute hepatitis A.[1,4] Anti-HAV IgM antibodies can always be detected at the onset of the disease, and usually disappear within 3-6 months but can be detected in some patients for a longer period of time.[1,4] Development of HAV IgM antibodies after vaccination is rare.[1]

Test principle

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

- 1st incubation: Pretreatment of 6 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 II 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 **cobas e** pack (M, R1, R2) is labeled as AHAVIGM.

M Streptavidin-coated microparticles, 1 bottle, 12.4 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 Anti-HAV Ab~Ru(bpy)$_3^{2+}$, 1 bottle, 21.0 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, 1 bottle, 21.0 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

AHAVIGM Cal1 Negative calibrator 1, 1 bottle of 0.67 mL:
Human serum, negative for anti-HAV IgM; preservative.

AHAVIGM Cal2 Positive calibrator 2, 1 bottle of 0.67 mL:
Anti-HAV IgM (human) approximately 5 U/mL (Roche units) in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use for health care professionals. Exercise the normal precautions required for handling all laboratory reagents.

Infectious or microbial waste:
Warning: handle waste as potentially biohazardous material. Dispose of waste according to accepted laboratory instructions and procedures.

Environmental hazards:
Apply all relevant local disposal regulations to determine the safe disposal.

Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

Warning

H317 May cause an allergic skin reaction.

Prevention:

P261 Avoid breathing dust/fume/gas/mist/vapours/spray.

P272 Contaminated work clothing should not be allowed out of the workplace.

P280 Wear protective gloves.

Response:

P333 + P313 If skin irritation or rash occurs: Get medical advice/attention.

P362 + P364 Take off contaminated clothing and wash it before reuse.

Disposal:

P501 Dispose of contents/container to an approved waste disposal plant.

Product safety labeling follows EU GHS guidance.

Contact phone: all countries: +49-621-7590

All human material should be considered potentially infectious. All products derived from human blood are 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 use assays that have been approved by the FDA or that are in compliance with the legal rules applicable to placing in vitro diagnostic medical devices for human use on the market in the European Union.

The calibrators (AHAVIGM Cal1 and AHAVIGM 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.

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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.[5,6]

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

Reagent handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in **cobas e** packs.

Calibrators:

The calibrators are supplied ready-for-use in bottles compatible with the system.

Unless the entire volume is necessary for calibration on the analyzer, 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 available via the **cobas** link.

Storage and stability

Store at 2-8 °C.

Do not freeze.

Store the **cobas e** pack **upright** in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability of the **cobas e** pack:	
unopened at 2-8 °C	up to the stated expiration date
on the analyzers	16 weeks

Stability of the calibrators:	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	16 weeks
on the analyzers 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-heparin, Na-heparin, K_2-EDTA, K_3-EDTA and Na-citrate plasma.

Criterion: Correct assignment of positive and negative samples. Samples with a COI (cutoff index) ≥ 1.0: ± 20 % recovery; samples with a COI < 1.0: ± 0.20 recovery.

Stable for 7 days at 15-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 5 times.

The sample types listed were tested with a selection of sample collection tubes or systems 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/collection system 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 and calibrators are at 20-25 °C prior to measurement.

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

The performance of the Elecsys Anti-HAV IgM assay has not been established with cadaveric samples or body fluids other than serum or plasma.

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, 16 x 0.67 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- REF 07299001190, Diluent Universal, 45.2 mL sample diluent
- General laboratory equipment
- **cobas e** analyzer

Additional materials for **cobas e** 402 and **cobas e** 801 analyzers:

- REF 06908799190, ProCell II M, 2 x 2 L system solution
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 07485409001, Reservoir Cup, 8 cups to supply ProCell II M and CleanCell M
- REF 06908853190, PreClean II M, 2 x 2 L wash solution
- REF 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- REF 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- REF 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 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.

Place the cooled (stored at 2-8 °C) **cobas e** pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the **cobas e** pack.

Calibrators:

Place the calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

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 AHAVIGM Cal1, AHAVIGM 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 8 weeks when using the same reagent lot
- after 28 days when using the same **cobas e** pack on the analyzer
- as required: e.g. quality control findings outside the defined limits

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 **cobas e** pack, 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.

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

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Calculation

The analyzer automatically calculates the cutoff based on the measurement of AHAVIGM Cal1 and AHAVIGM 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

Numeric result	Result message	Interpretation/further steps
COI < 1.0	Non-reactive	Negative for HAV IgM-specific antibodies
COI ≥ 1.0	Reactive	Positive for HAV IgM-specific antibodies

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

Compound	Concentration tested
Bilirubin	≤ 855 µmol/L or ≤ 50 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 1000 mg/dL
Biotin	≤ 205 nmol/L or ≤ 50 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL

Criterion: Samples with a COI ≥ 1.0: ± 20 % recovery; samples with a COI < 1.0: ± 0.20 recovery.

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.

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

Pharmaceutical substances
In vitro tests were performed on 16 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.

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.[7,8,9]

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, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

cobas e 402 and **cobas e** 801 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean COI	SD COI	CV %	SD COI	CV %
HS[c], negative	0.317	0.005	1.6	0.008	2.5
HS, weakly positive	1.12	0.020	1.8	0.025	2.2
HS, positive	3.28	0.073	2.2	0.091	2.8
PC[d] Anti-HAV IgM 1	0.310	0.004	1.3	0.006	2.0
PC Anti-HAV IgM 2	1.92	0.054	2.8	0.101	5.3

c) HS = human serum

d) PC = PreciControl

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 %.

References

1 Hollinger FB, Emerson SU. Hepatitis A virus. In: Fields Virology, Knipe DM, Howley PM (eds), 2007 5th edition, Lippincott Williams and Wilkins, Philadelphia, USA. Chapter 27, pp911-947.

2 Lu L, Ching KZ, de Paula SV, et al. Characterization of the complete genomic sequence of genotype II hepatitis A virus (CF53/Berne isolate). J Gen Virol 2004;855:2943-2952.

3 Martin A, Lemon SM. Hepatitis A virus: from discovery to vaccines. Hepatology 2006 Feb;43(2 Suppl 1):S164-172.

4 Wasley A, Fiore A, Bell BP. Hepatitis A in the era of vaccination. Epidemiol Rev 2006;28:101-111.

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

6 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.

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

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

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

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.

Any serious incident that has occurred in relation to the device shall be reported to the manufacturer and the competent authority of the Member State in which the user and/or the patient is established.

The Summary of Safety & Performance Report can be found here: https://ec.europa.eu/tools/eudamed

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard (for USA: see dialog.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 0123

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