Elecsys Anti-HAV II

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REF		Σ	SYSTEM
08086664190	08086664500	300	**cobas e** 402 **cobas e** 801

English

System information

Short name	ACN (application code number)
AHAV 2	10156

Intended use

Immunoassay for the in vitro qualitative detection of total antibodies (IgG and IgM) to the hepatitis A virus (HAV) in human serum and plasma. The assay is used as an aid to detect a past or existing hepatitis A infection or used to determine the presence of antibody response to HAV in vaccine recipients.

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 because the virus does not persist in the organism.[1,3]

Total anti-HAV (anti-HAV IgM and IgG) is positive at the onset of symptoms and due to the presence of IgM.[4] After natural infection, anti-HAV IgG can usually be detected early in the course of infection and remains detectable throughout a person's lifetime conferring protection against the disease if the organism is reinfected.[4,5]

Vaccines against HAV and combined vaccines against hepatitis A and B are available today.[3,4] Anti-HAV IgG can be detected approximately 2 weeks after vaccination against HAV. In the case of complete immunization, protection usually lasts for many years. To define a protective antibody response, clinical vaccine studies typically used anti-HAV levels of > 20 IU/L, while some studies have used levels of > 10 IU/L.[5,6,7] A positive anti-HAV result indicates immune protection. However, after vaccination, persons who are anti-HAV negative (< 20 IU/L) might nevertheless have protective levels of antibody. The absolute lower limit of anti-HAV required to prevent HAV infection has not been defined. In vitro studies using cell-culture-derived virus indicate that low levels of antibody (e.g. < 20 IU/L) can be neutralizing.[8]

Assays to detect anti-HAV antibodies are used to determine an existing or past HAV infection or to observe the immune response after HAV vaccination.[1]

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 12 µL of sample; the anti-HAV in the sample binds the added HAV antigen.
- 2nd incubation: After addition of biotinylated antibodies and ruthenium complex[a]-labeled antibodies specific for HAV antigen, together with streptavidin-coated microparticles, the still-free binding sites on the HAV antigens become occupied. The entire complex 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 AHAV 2.

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

R1 HAV Ag, 1 bottle, 21 mL:
HAV Ag (cell culture) 28 U/mL (Roche units); TRIS buffer 20 mmol/L, pH 7.2; preservative.

R2 Anti-HAV Ab~biotin; anti-HAV Ab~$Ru(bpy)_3^{2+}$, 1 bottle, 19 mL:
Biotinylated monoclonal anti-HAV antibody (mouse) 0.25 µg/mL; monoclonal anti-HAV antibody (mouse) labeled with ruthenium complex 0.65 µg/mL; TRIS buffer 20 mmol/L, pH 7.2; preservative.

AHAV 2 Cal1 Negative calibrator 1, 1 bottle of 1.0 mL:
Human serum; preservative.

AHAV 2 Cal2 Positive calibrator 2, 1 bottle of 1.0 mL:
Anti-HAV (human) approximately 60 IU/L 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.

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

Product safety labeling follows EU GHS guidance.

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.

However, as no 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 (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 first opening	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, ACD, CPD, CP2D, CPDA and Na-citrate plasma.

Plasma tubes containing separating gel can be used.

Criterion: Correct assignment of positive and negative samples. Samples with a COI (cutoff index) > 1.0: ± 20 % recovery compared to serum reference; samples with a COI ≤ 1.0: ± 0.20 COI recovery compared to serum reference.

Stable for 6 days at 20-25 °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 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 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 II assay has not been established with cadaveric samples or body fluids other than serum and plasma.

Materials provided

- See “Reagents – working solutions” section for reagents.
- 2 x 6 bottle labels

Materials required (but not provided)

- REF 08086672190, PreciControl Anti-HAV II, for 8 x 1.3 mL
- REF 11776576322, CalSet Vials, 2 x 56 empty bottles with snap-caps
- 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: The Elecsys Anti-HAV II assay is traceable to the “Second International Standard for Anti-Hepatitis A, immunoglobulin, human", NIBSC code 97/646 of the NIBSC (National Institute for Biological Standards and Control) via method comparison to the first generation Elecsys Anti-HAV assay as reference.

Calibration frequency: Calibration must be performed once per reagent lot using AHAV 2 Cal1, AHAV 2 Cal2 and fresh reagent (i.e. not more than 24 hours since the **cobas e** pack 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

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Quality control

For quality control, use PreciControl Anti-HAV II.

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.

Calculation

The analyzer automatically calculates the cutoff based on the measurement of AHAV 2 Cal1 and AHAV 2 Cal2.

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

Interpretation of the results

Numeric result	Result message	Interpretation
COI > 1.0	Non-reactive	Negative for HAV-specific antibodies
COI ≤ 1.0	Reactive	Positive for HAV-specific antibodies

The cutoff of the Elecsys Anti-HAV II assay was determined in a large cohort of blood donors and hospitalized patients using the first generation Elecsys Anti-HAV assay, which has a cutoff of 20 IU/L, as the reference. The cutoff of the Elecsys Anti-HAV II assay thus corresponds to 20 IU/L.

COI values ≤ 1.0 indicate an existing or past hepatitis A infection or the presence of anti-HAV antibodies after hepatitis A vaccination.

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	≤ 1129 µmol/L or ≤ 66 mg/dL
Hemoglobin	≤ 0.621 mmol/L or ≤ 1000 mg/dL
Intralipid	≤ 2000 mg/dL
Biotin	≤ 573 nmol/L or ≤ 140 ng/mL
Rheumatoid factors	≤ 1400 IU/mL
IgG	≤ 7.0 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1.0 g/dL

Criterion:
Samples > 1.0 COI ± 20 % recovery
Samples ≤ 1.0 COI ± 0.20 COI 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.

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

In rare cases, interference due to extremely high titers of antibodies to analyte-specific antibodies, streptavidin or ruthenium can occur. These effects are minimized by suitable test design.

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

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 %
Human serum 1	1.41	0.012	0.9	0.028	2.0
Human serum 2	1.13	0.012	1.1	0.025	2.2
Human serum 3	0.932	0.010	1.1	0.023	2.4
Human serum 4	0.645	0.009	1.5	0.023	3.5
Human serum 5	0.006	0.0001	1.3	0.0001	2.4
PC[b] Anti-HAV II 1	1.30	0.012	0.9	0.027	2.1
PC Anti-HAV II 2	0.318	0.005	1.6	0.010	3.0

b) PC = PreciControl

Analytical specificity

No cross-reactions with HBV, HCV, HIV, CMV, EBV, HSV, Toxoplasma gondii, Rubella, Mumps/Rubeola, Parvovirus B19 and Treponema pallidum were observed.
Measurements were performed on each of the pathogens listed above using a total of 120 human serum or plasma which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (ANA).

Clinical data

In the clinical studies, conducted to assess the relative sensitivity and relative specificity of the assay, samples from various international sources were used.

Clinical sensitivity

The relative sensitivity was found to be 100 % in samples from subjects vaccinated against HAV, acutely infected subjects and subjects who had recovered from a natural HAV infection.

	N	Sensitivity %	95 % CI[c] (2-sided) %
Subjects vaccinated against hepatitis A	238	100	98.45-100
Subjects with acute hepatitis A infection	234	100	98.44-100
Subjects recovered from hepatitis A infection	256	100	98.57-100

c) CI = confidence interval

Clinical specificity

A total of 874 confirmed anti-HAV negative samples from subjects with routine requests for anti-HAV testing and 580 samples confirmed anti-HAV negative from blood donors were tested with the Elecsys Anti-HAV II assay.

	N[d]	Specificity %	95 % CI (2-sided) %
Blood donors	577	99.48	98.49-99.89
Subjects with routine request for anti-HAV testing	871	99.66	99.00-99.93

d) Number of subjects with a negative Elecsys Anti-HAV II test result

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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 Tilzey, AJ, Palmer SJ, Barrow S, et al. Clinical trial with inactivated hepatitis A vaccine and recommendations for its use. BMJ 1992;304:1272-1276.

6 Briem H, Safary A. Immunogenicity and Safety in Adults of Hepatitis A Virus Vaccine Administered as a Single Dose With a Booster 6 Months Later. J Med Virol 1994;44:443-445.

7 Ambrosch F, Finkel B, Herzog C, et al. Rapid Antibody Response after Vaccination with a Virosomal Hepatitis A Vaccine. Infection 2004;32:149-152.

8 Lemon SM, Binn LN. Serum Neutralizing Antibody Response to Hepatitis A Virus. J Infect Dis 1983;148:1033-1039.

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.

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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