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Anti-HAV

cobas®

Total antibodies (IgM and IgG) to the hepatitis A virus

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
04854977 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of total antibodies to the hepatitis A virus in human serum and plasma. The anti-HAV assay is used as an aid to detect a past or existing hepatitis A infection, and to observe the immune response after HAV vaccination.

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

Summary

The hepatitis A virus is an 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]

The hepatitis A virus is one of the most common causes (10-20 %) of a fulminating course of viral hepatitis.[3]

Total anti-HAV is positive at the onset of a hepatitis A infection (IgM). After natural infection, anti-HAV IgG antibodies can usually be detected lifelong and provide protection against the disease if the organism is reinfected.[4]

Vaccines against hepatitis A and combined vaccines against hepatitis A and B are available today.[5] Upon vaccination against hepatitis A, anti-HAV IgG antibodies can be detected after 2 weeks. In the case of complete immunization, protection usually lasts for years.[6] There is no limit value to define immune protection but anti-HAV values > 10-20 IU/L are generally considered to be protective against infection.

Assays to detect anti-HAV antibodies are used to determine an existing or past hepatitis A infection. They are also used to observe the immune response after HAV vaccination.

Test principle

Competition principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample; the sample anti-HAV 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/ProCell M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

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-HAV.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:

Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HAV Ag (gray cap), 1 bottle, 7 mL:

HAV Ag (human) 40 U/mL (Roche units); HEPES[b] buffer 50 mmol/L, pH 7.2; preservative.

R2 Anti-HAV Ab~biotin; anti-HAV Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:

Biotinylated monoclonal anti-HAV antibody (mouse) 0.25 µg/mL; monoclonal anti-HAV antibody (mouse) labeled with ruthenium complex 0.15 µg/mL; HEPES buffer 50 mmol/L, pH 7.2; preservative.

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

A-HAV Cal1 Negative calibrator 1 (white cap), 2 bottles (lyophilized) for 1.0 mL each:

Anti-HAV negative human serum; preservative.

A-HAV Cal2 Positive calibrator 2 (black cap), 2 bottles (lyophilized) for 1.0 mL each:

Anti-HAV (human) approx. 46 IU/L 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-HAV Cal1 and A-HAV 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 applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HAV (A-HAV Cal2) and the HAV Ag (human; R1) 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.[7,8]

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

Reagent handling

The reagents in the kit are ready for use (except for A-HAV Cal1 and A-HAV Cal2) and are supplied in bottles compatible with the system.

Calibrators

Carefully dissolve the contents of one bottle by adding exactly 1.0 mL of distilled or deionized water and allow to stand closed for 15 minutes to reconstitute. Mix carefully, avoiding foam formation.

Transfer the reconstituted calibrators into the supplied empty labeled snap-cap bottles.

Elecsys 2010 and **cobas e** 411 analyzers: The calibrators should only be left on the analyzers 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 reconstituted calibrators into empty snap-cap bottles

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(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.

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 at 20-25 °C	7 days or 4 weeks when stored alternatively in the refrigerator and on the analyzer, with the total time onboard on the analyzer not exceeding 40 hours

Stability of the calibrators	
lyophilized calibrators	up to the stated expiration date
reconstituted calibrators at 2-8 °C	6 weeks
on Elecsys 2010 and **cobas e** 411 at 20-25 °C	up to 5 hours
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	use once only

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 serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

Stable for 7 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen 6 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 frozen 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 6 bottle labels
- 4 empty labeled snap-cap bottles

Materials required (but not provided)

- REF 04855043190, PreciControl Anti-HAV, for 2 x 4 mL each of PreciControl Anti-HAV 1 and 2
- REF 11361252122, Diluent Hepatitis A, 2 x 15 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty bottles with snap-caps
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer
- Distilled or deionized water

Accessories for Elecsys 2010 and **cobas e** 411 analyzers:

- 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, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

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 Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, 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.

Bring the cooled reagents to approx. 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 reconstituted 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 the "Second International Standard for Anti-Hepatitis A, Immunoglobulin, Human, NIBSC code: 97/646" of the NIBSC (National Institute for Biological Standards and Control).

Calibration frequency: Calibration must be performed once per reagent lot using A-HAV Cal1, A-HAV Cal2 and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer). 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 outside the defined limits
- more frequently when this is required by pertinent regulations

Quality control

For quality control, use PreciControl Anti-HAV.

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.

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

Note:

Always consider the value sheet contained in the kit of the assay/PreciControl and make sure whether target values for specific assay/PreciControl combinations need to be entered manually.

Calculation

The analyzer automatically calculates the analyte concentration of each sample in IU/L.

Interpretation of the results:

Samples with concentrations < 20 IU/L are not reactive in the Elecsys Anti-HAV assay.

Samples with concentrations ≥ 20 IU/L are reactive in the Elecsys Anti-HAV assay.

Concentrations ≥ 20 IU/L indicate an exisiting or past hepatitis A infection or the presence of anti-HAV antibodies after hepatitis A vaccination.

Important! The anti-HAV values measured may differ depending on the assay method used. The test result obtained of an individual sample can hence vary when using assays of different manufacturers. If there is a change in the assay procedure during the monitoring of vaccination protection, then the anti-HAV values obtained upon changing over to the new method must be confirmed by parallel measurements by both methods.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 855 µmol/L or < 50 mg/dL), hemolysis (Hb < 0.745 mmol/L or < 1.2 g/dL), lipemia (Intralipid < 1000 mg/dL) and biotin (< 205 nmol/L or < 50 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

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 1600 IU/mL.

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.

Important!

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

Vaccination against hepatitis A should be considered where there is any uncertainty, and in particular if the test results borderline the cutoff (20 IU/L).

Limits and ranges

Measuring range

3.00-60 IU/L (defined by the lower detection limit and the maximum of the master curve). Values below the lower detection limit are reported as < 3.00 IU/L. Values above the measuring range are reported as > 60 IU/L or must be diluted.

Lower limits of measurement

Lower detection limit of the test

Lower detection limit: < 3.0 IU/L

The lower detection limit represents the lowest measurable analyte level that can be distinguished from zero. It is calculated as the value lying two standard deviations above that of the lowest standard (master calibrator, standard 1 + 2 SD, repeatability study, n = 21).

Dilution

Samples with anti-HAV concentrations above the measuring range can be diluted with Diluent Hepatitis A. The concentration of the diluted sample must be > 20 IU/L. Multiply the results by the dilution factor.

Samples containing anti-HAV IgM may, in isolated cases, show non-linear dilution behavior.

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:

Elecsys 2010 and **cobas e** 411 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean IU/L	SD IU/L	CV %	Mean IU/L	SD IU/L	CV %
HS[c], negative	< 3.0	0.29	-	< 3.0	0.69	-
HS, borderline cutoff	17.6	0.22	1.2	18.4	0.74	4.0
HS, positive	41.9	0.48	1.2	42.3	1.23	2.9
PC[d] A-HAV1	20.9	0.40	1.9	21.7	0.81	3.7
PC A-HAV2	35.8	0.84	2.3	36.5	1.02	2.8

c) HS = human serum

d) PC = PreciControl

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability			**Intermediate precision**		
Sample	Mean IU/L	SD IU/L	CV %	Mean IU/L	SD IU/L	CV %
HS, negative	< 3.0	0.34	-	< 3.0	0.83	-
HS, borderline cutoff	21.8	0.11	0.5	29.2	0.89	3.0
HS, positive	55.2	0.30	0.5	51.3	1.12	2.2
PC A-HAV1	21.1	0.17	0.8	21.0	0.57	2.7
PC A-HAV2	35.2	0.23	0.7	34.8	0.56	1.6

Analytical specificity

No cross-reactions with HBV, HCV, HIV, CMV, EBV*, HSV, Toxoplasma gondii, Rubella*, and Treponema pallidum* were observed.

Measurements were performed using a total of 140 anti-HAV negative serum and plasma samples which were positive for antibodies to the above-mentioned pathogens or contained autoantibodies (ANA, AMA).

* 1 sample out of each group was weakly positive.

Clinical sensitivity

In 97 samples of patients with an existing (anti-HAV IgM positive) or past HAV infection, HAV antibodies (concentration > 20 IU/L) were detected using the Elecsys Anti-HAV test. 165 samples from 46 HAV-vaccinated persons were tested using the Elecsys Anti-HAV test and a commercially available comparison test. In all samples the anti-HAV antibody concentration found was above the respective limit.

Clinical specificity

To investigate the specificity, a total of 1301 samples of non-selected blood donors, hospitalized patients, pregnant women, and dialysis patients where

no HAV infection was indicated were tested using the Elecsys Anti-HAV test and a comparison test. Using the Elecsys Anti-HAV assay, 1286 gave results < 20 IU/L. The specificity in this study is 98.85 %. The 95 % confidence range is 98.11-99.35 %.

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

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

5 Ambrosch F, Wiedermann G, André FE, et al. Clinical and Immunological Investigation of a New Combined Hepatitis A and Hepatitis B Vaccine. J Med Virol 1994;44:452-456.

6 Dobler G, Nitschko H, Frösner GG, et al. Hepatitis A: Medizinische Bedeutung, Klinik, Diagnostik, Aussagewert diagnostischer Verfahren. In: Frösner G (Hrsg.). Moderne Hepatitisdiagnostik, Kilian Verlag 2001;19-30, 2nd edition.

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

8 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.

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

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