

Elecsys Anti-HCV II

cobas®

| | | | |
|-------------|-------------|-----|--------------------|
| 07026889119 | 07026889500 | 300 | SYSTEM |
| | | | cobas e 801 |

English

System information

| | |
|------------|-------------------------------|
| Short name | ACN (application code number) |
| AHCV 2 | 10104 |

Intended use

The Elecsys Anti-HCV II assay is an in vitro diagnostic test for the qualitative detection of antibodies to hepatitis C virus (HCV) in human serum and plasma. This assay is indicated as an aid in the diagnosis of infection with HCV. This assay is also indicated as a donor screening test to detect antibodies to HCV in serum or plasma specimens from individual human blood donors. This assay may also be used to detect antibodies to HCV in serum and plasma specimens to screen donors of cells (excluding blood cells and derivatives), tissues and organs intended for transplantation.

The electrochemiluminescence immunoassay "ECLIA" is intended for use on the **cobas e 801** immunoassay analyzer.

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 07026889190 for the Elecsys Anti-HCV II assay. The last 3 digits -190 have been replaced by -119 for logistic purposes

Summary

The hepatitis C virus (HCV), first identified in 1989, is a leading cause of liver disease and a major healthcare concern with over 170 million persons (roughly 3 % of the human population), infected worldwide.^{2,3} The highest prevalence is found in Africa, the Eastern Mediterranean and Asian regions.^{3,4} HCV is a member of the Flaviviridae family and has a single-stranded, positive-sense RNA genome.⁵ Currently over 67 subtypes have been identified and these have been classified into 7 genotypes (1-7).⁶

Due to the high rate of asymptomatic infections, clinical diagnosis is difficult and screening assays are of major importance.⁷ Infection with HCV can lead to acute and chronic hepatitis disease. Approximately 70-85 % of HCV infections progress to chronic disease, although this varies according to patient gender, age, race and immune status.^{5,8} Chronic HCV infection may lead to cirrhosis and hepatocellular carcinoma,⁹ therefore, early anti-HCV detection is the first step in the management of chronic hepatitis and in the selection of patients needing treatment.⁷ HCV infection can be detected by measuring the amount of HCV RNA, alanine aminotransferase (ALT) and HCV-specific immunoglobulins (anti-HCV) in patient serum or plasma samples. This can also indicate if the infection is acute or chronic.^{5,8}

Anti-HCV antibody tests are used alone or in combination with other tests (e.g. HCV RNA) to detect an infection with HCV and to identify blood and blood products of individuals infected with HCV. The Elecsys Anti-HCV II assay is a third-generation test.^{10,11} The Elecsys Anti-HCV II assay uses peptides and recombinant proteins representing HCV core, NS3 and NS4 antigens for the determination of anti-HCV antibodies.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 30 µL of sample, a reagent containing biotinylated HCV-specific antigens and a reagent containing HCV-specific antigens labeled with a ruthenium complex^{a)} react to form a sandwich complex.
- 2nd incubation: After addition of streptavidin-coated microparticles, the 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)₃²⁺)

Reagents - working solutions

The **cobas e** pack (M, R1, R2) is labeled as AHCV 2.



300

- M Streptavidin-coated microparticles, 1 bottle, 14.1 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 HCV-specific antigens-biotin, 1 bottle, 14.8 mL:
Biotinylated HCV-specific antigens, HEPES^{b)} buffer, pH 7.4; preservative.
- R2 HCV-specific antigens-Ru(bpy)₃²⁺, 1 bottle, 14.8 mL:
HCV-specific antigens labeled with ruthenium complex ≥ 0.3 mg/L, HEPES buffer, pH 7.4; preservative.

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

- AHCV 2 Cal1 Negative calibrator 1, 1 bottle of 1.3 mL:
Human serum negative for anti-HCV Ab; preservative.
- AHCV 2 Cal2 Positive calibrator 2, 1 bottle of 1.3 mL:
Human serum positive for anti-HCV Ab; preservative. Non-reactive for HBsAg, anti-HIV 1/2.

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.

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 (AHCV 2 Cal1 only) 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-HCV (AHCV 2 Cal2) was 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.^{12,13}

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

The Elecsys Anti-HCV II assay has a high dilution sensitivity. Avoid any sample cross-contamination during sample pre-analytics.

Reagent handling

The pouch should remain sealed until immediately prior to use.

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 cobas e 801 analyzer | 31 days |

Elecsys Anti-HCV II



| Stability of the calibrators: | |
|--|----------------------------------|
| unopened at 2-8 °C | up to the stated expiration date |
| after opening at 2-8 °C | 8 weeks |
| on the cobas e 801 analyzer 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, K₂-EDTA, K₃-EDTA, ACD, CPDA and Na-citrate plasma.

Plasma tubes containing separating gel can be used.

Criterion: Correct assignment of positive and negative samples within a recovery of 80-120 % of serum value.

CPD and CP2D plasma.

Criterion: Correct assignment of positive and negative samples within a recovery of 80-130 % of serum value.

Stability:

For living patients and donor specimens obtained while the donor's heart is still beating: Stable for 7 days at 20-25 °C, 14 days at 2-8 °C, 3 months at -20 °C (± 5 °C). The samples may be frozen 6 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 manufacturer.

Centrifuge samples containing precipitates before performing the assay.

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-HCV 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](#) 03290379190, PreciControl Anti-HCV, 16 x 1.3 mL
- General laboratory equipment
- cobas e** 801 analyzer

Accessories for the **cobas e** 801 analyzer:

- [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 Cups, 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

No internationally accepted standard for anti-HCV exists.

Calibration frequency: Calibration must be performed once per reagent lot using AHCV 2 Cal1, AHCV 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 12 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

Range for electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (AHCV 2 Cal1): 400-3000
Positive calibrator (AHCV 2 Cal2): 25000-350000

Quality control

For quality control, use PreciControl Anti-HCV.

In addition, other suitable control material can be used.

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 AHCV 2 Cal1 and AHCV 2 Cal2.

The result of a sample is given either as reactive, borderline 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 ^(c) < 0.9 | Non-reactive | Negative for anti-HCV, no further testing needed. |

Elecsys Anti-HCV II

| Numeric result | Result message | Interpretation/ further steps |
|---------------------------|----------------|---|
| COI ≥ 0.9 to < 1.0 | Borderline | All initially reactive or borderline samples should be retested in duplicate using the Elecsys Anti-HCV II assay. |
| COI ≥ 1.0 | Reactive | |

c) COI = cutoff index

| Retest result | Final result/ interpretation | Further steps |
|--|---------------------------------|---|
| One or both of the duplicate retests have a COI ≥ 0.9 . | Repeatedly reactive | Confirmation via supplemental methods (e.g. immunoblot or detection of HCV RNA). If one or both measurements remain borderline the analysis of a follow-up sample is recommended. |
| Both of the duplicate retests have a COI < 0.9 . | Negative for anti-HCV | No further testing needed. |

Retesting of samples with an initial cutoff index ≥ 0.9 can be automatically performed (see section "cobas e flows").

cobas e flows

cobas e flows are procedures programmed into the system to enable a fully automated sequence of measurements and the calculation of assay combinations to perform decision algorithms.

A cobas e flow is available to perform a repetition of measurements in duplicate automatically for samples with an initial cutoff index ≥ 0.9 . Both sub-results and the overall result message will be reported.

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 | $\leq 1129 \mu\text{mol/L}$ or $\leq 66 \text{ mg/dL}$ |
| Hemoglobin | $\leq 0.621 \text{ mmol/L}$ or $\leq 1000 \text{ mg/dL}$ |
| Intralipid | $\leq 2000 \text{ mg/dL}$ |
| Biotin | $\leq 172 \text{ nmol/L}$ or $\leq 42 \text{ ng/mL}$ |
| Rheumatoid factors | $\leq 1200 \text{ IU/mL}$ |
| Albumin | $\leq 7 \text{ g/dL}$ |
| IgG | $\leq 7 \text{ g/dL}$ |
| IgA | $\leq 1.6 \text{ g/dL}$ |
| IgM | $\leq 1 \text{ g/dL}$ |

Criterion: Samples with a COI ≥ 1.0 : $\pm 20\%$ recovery; samples with a COI < 1.0 : ± 0.2 COI recovery.

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

Pharmaceutical substances

In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs used in hepatitis C therapy were tested. No interference with the assay was found.

Special drugs

| Drug | Concentration tested |
|-----------------------|--------------------------|
| Peginterferon alfa-2a | $\leq 0.18 \text{ mg/L}$ |

| Drug | Concentration tested |
|-----------------|----------------------|
| Interferon alfa | 20 IU/L |
| Ribavirin | 1200 mg/L |

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

Studies have been performed to assess the high-dose hook effect. Out of 765 positive samples no false negative result was found. Occurrence of high-dose hook effect cannot be completely excluded.

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

Due to a long time period from infection to seroconversion, negative anti-HCV test results may occur during early infection. If acute hepatitis C infection is suspected, measuring of HCV RNA by reverse transcriptase polymerase chain reaction (RT-PCR e.g. by COBAS AmpliPrep/COBAS TaqMan HCV Qualitative Test, v2.0) may give evidence of HCV infection.

The detection of anti-HCV antibodies indicates a present or past infection with HCV, but does not differentiate between acute, chronic or resolved infection. It is recognized within the scientific community that presently available methods for anti-HCV detection are not sensitive enough to detect all potentially infectious units of blood or possible cases of HCV infection. The antibody concentration may be beneath the detection limit of this assay or the patient's antibodies do not react with the antigens used in this test. In addition, non-specific results cannot be ruled out with the Elecsys Anti-HCV II assay.

Specific performance data

Representative performance data on the analyzer is 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 801 analyzer | | | | | |
|-----------------------------|-------------|-----------------------------|---------|---|---------|
| Sample | Mean COI | Repeatability ^{d)} | | Intermediate precision ^{e)} | |
| | | SD COI | CV % | SD COI | CV % |
| HS ^{f)} , negative | 0.040 | 0.001 | 2.3 | 0.001 | 2.5 |
| HS, weakly positive | 1.16 | 0.012 | 1.1 | 0.017 | 1.4 |
| HS, positive | 21.3 | 0.144 | 0.7 | 0.247 | 1.2 |
| PC ^{g)} Anti-HCV 1 | 0.051 | 0.001 | 2.0 | 0.001 | 2.1 |
| PC Anti-HCV 2 | 3.74 | 0.034 | 0.9 | 0.056 | 1.5 |

d) Repeatability = within-run precision

e) Intermediate precision = within-laboratory precision

f) HS = Human serum

g) PC = PreciControl

Analytical specificity

1037 samples containing potentially interfering substances or derived from high-risk groups were tested with the Elecsys Anti-HCV II assay comprising specimens:

- containing antibodies against HBV, HAV, HEV, EBV, CMV, HSV, HIV, VZV, Parvovirus, Mumps, Dengue, tick-borne encephalitis virus (TBEV), Rubella, Toxoplasma gondii, Treponema pallidum
- containing autoantibodies and elevated titers of rheumatoid factor, IgG, IgM or IgA antibodies
- positive for HBsAg and E. coli
- after vaccination against HBV and Influenza
- non-viral liver diseases
- alcoholic liver disease

Elecsys Anti-HCV II



- high-risk groups: hemophiliacs, homosexuals and intravenous drug abusers

| | N | Elecsys Anti-HCV II reactive | Positive or indeterminate by immunoblot | Negative by immunoblot |
|---|------|------------------------------|---|------------------------|
| Specimens containing potentially interfering substances | 1037 | 59 | 58 positive | 1 ^{h)} |

h) EBV IgM positive patients: 1 out of 69 samples

Clinical sensitivity

Of 765 samples from HCV infected patients with different stages of disease and infected with different HCV genotypes (type 1, 2, 3, 4, 5 and 6), all samples were found to be reactive with the Elecsys Anti-HCV II assay.

| Group | N | Reactive |
|---|-----|----------|
| HCV infected persons with different stages of disease | 224 | 224 |
| HCV genotypes (type 1, 2, 3, 4, 5, 6) | 541 | 541 |

In the above study the diagnostic sensitivity was 100 %. The 95 % lower confidence limit was 99.61 %.

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HCV II assay has been shown by testing 60 commercial seroconversion panels. Elecsys Anti-HCV II detected more positive bleedings than all other registered anti-HCV assays tested and was more sensitive in the recognition of early HCV infection than Elecsys Anti-HCV and the other registered anti-HCV screening assays.

Clinical specificity

In a group of randomly selected European blood donors the specificity of the Elecsys Anti-HCV II assay was 99.85 % (RR^{l)}). The 95 % confidence interval (2-sided) was 99.73-99.93 %.

The diagnostic specificity of the Elecsys Anti-HCV II assay in a group of hospitalized patients was 99.66 %. The 95 % confidence interval (2-sided) was 99.41-99.82 %.

| | N | Elecsys Anti-HCV II IR ^{l)} COI ≥ 1 | Elecsys Anti-HCV II RR COI ≥ 1 | Positive or indeterminate by immunoblot and/or HCV RNA |
|-----------------------|------|--|--------------------------------|--|
| European blood donors | 6850 | 15 | 15 | 2 confirmed positive, 3 indeterminate |
| Hospitalized patients | 3922 | 153 ^{j)} | 152 ^{k)} | 128 confirmed positive, 8 indeterminate |
| Dialysis patients | 731 | 19 | 18 | 12 confirmed positive |
| Pregnant women | 629 | 3 | 3 | 2 confirmed positive |

i) IR = Initially Reactive

j) 4 (positive) samples had to be excluded from calculation due to "qns" for immunoblot analysis; qns = quantity not sufficient

k) 4 (positive) samples had to be excluded from calculation due to "qns" for immunoblot analysis

l) RR = Repeatedly Reactive

References

- Choo QL, Kuo G, Weiner AJ, et al. Isolation of a cDNA clone derived from a blood-borne non-A, non-B viral hepatitis genome. *Science* 1989;244:359-362.
- Lavanchy D. The global burden of hepatitis C. *Liver Int* 2009;29(s1):74-81.
- Hepatitis C WHO report WHO/SCD/SCR/LYO/2003 <http://www.who.int/csr/disease/hepatitis/Hepc.pdf>
- Hoofnagle JH. Course and outcome of hepatitis C. *Hepatology* 2002;36:21-29.
- Smith DB, Bukh J, Kuiken C, et al. Expanded classification of hepatitis C virus into 7 genotypes and 67 subtypes: updated criteria and assignment web resource. *Hepatology* 2013;59:318-327.
- Strader DB, Wright T, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C. *Hepatology* 2004;39(4):1147-1171.
- Lemon SM, Walker CM, Alter MJ, et al. *Fields Virology*. Lippincott Williams and Wilkins, Philadelphia. Hepatitis C virus; 2007:1253-1304.
- Di Bisceglie AM. Hepatitis C and hepatocellular carcinoma. *Hepatology* 1997;26(Suppl 1):34-38.
- Couroucé A-M. Development of Screening and Confirmation Tests for Antibodies to Hepatitis C Virus. In: Reesink HW (ed.): *Hepatitis C Virus*. *Curr Stud Hematol Blood Transf*. Basel, Karger, 1998;62:64-75.
- Vernelen K, Claeys H, Verhaert H, et al. Significance of NS3 and NS5 antigens in screening for HCV antibody. *The Lancet* 1994;343(8901):853.
- Occupational Safety and Health Standards: Bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
- 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):

| | |
|--|---|
| | Contents of kit |
| | Analyzers/Instruments on which reagents can be used |
| | Reagent |
| | Calibrator |
| | Volume after reconstitution or mixing |
| | Global Trade Item Number |

COBAS, COBAS E, ELECSYS, PRECICONTROL, AMPLIPREP and TAQMAM are trademarks of Roche. INTRALIPID is a trademark of Fresenius Kabi AB.

All other product names and trademarks are the property of their respective owners.

Additions, deletions or changes are indicated by a change bar in the margin.

© 2018, Roche Diagnostics



Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com

