Elecsys Anti-HCV II

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REF		Σ	SYSTEM
			cobas e 411
08837031190	08837031501	200	**cobas e** 601
			cobas e 602

For use in the USA only

System information

For **cobas e** 411 analyzer: test number 2150
For **cobas e** 601 and **cobas e** 602 analyzers: Application Code Number 503

Warning

- Federal law restricts this device to sale by or on the order of a physician.
- Assay performance characteristics have not been established in populations of immunocompromised or immunosuppressed patients.
- This assay has not been FDA licensed for the screening of blood, plasma, and cell or tissue donors.

Intended use

Immunoassay for the in vitro qualitative detection of antibodies to hepatitis C virus (HCV) in human adult and pediatric (ages 18 months through 21 years) serum and plasma (potassium EDTA, lithium heparin, sodium heparin, and sodium citrate). Assay results, in conjunction with other laboratory results and clinical information, may be used to aid in the presumptive diagnosis of HCV infection in persons with signs and symptoms of hepatitis and in persons at risk for hepatitis C infection. The test does not determine the state of infection or associated disease.

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

Summary

The hepatitis C virus (HCV), first identified in 1989, is a leading cause of liver disease and a major healthcare concern. The most recent estimates of disease burden show an increase in seroprevalence over the last 15 years to 2.8 %, equating to > 185 million infections worldwide.[1] HCV is a member of the Flaviviridae family and has a single-stranded, positive-sense RNA genome.[2] Currently over 60 subtypes have been identified and these have been classified into 7 genotypes (1-7).[3]

Due to the high rate of asymptomatic infections, clinical diagnosis is difficult and diagnostic assays are of major importance.[4] 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.[2,5] Chronic HCV infection may lead to cirrhosis and hepatocellular carcinoma.

Anti-HCV antibody tests are used in combination with other tests (e.g. HCV-RNA) to detect an infection with hepatitis C virus. The Elecsys Anti-HCV II assay is a third-generation test.[6,7] The Elecsys Anti-HCV II assay uses peptides and recombinant antigens representing core, NS3 and NS4 proteins for the determination of anti-HCV antibodies.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, 55 µL of a reagent containing biotinylated HCV-specific antigens and 55 µL of 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/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-HCV II.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 12 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HCV-specific antigens~biotin (gray cap), 1 bottle, 18 mL: Biotinylated HCV-specific antigens, HEPES[b)] buffer, pH 7.4; preservative.

R2 HCV-specific antigens~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 18 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

A-HCV II Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each: Human serum, preservative.

A-HCV II Cal2 Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each: Human serum positive for anti-HCV Ab; preservative. Non-reactive for HBsAg, anti-HIV 1/2.

Precautions and warnings

For in vitro diagnostic use for healthcare 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.

H319 Causes serious eye irritation.

Prevention:

P261 Avoid breathing mist or vapours.

P280 Wear protective gloves/ eye protection/ face protection.

Response:

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

P337 + P313 If eye irritation persists: Get medical advice/attention.

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

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Disposal:

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

Product safety labeling follows EU GHS guidance based on the FDA recognized guideline (ISO20417: 2021).

Contact phone: 1-800-428-2336

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 (A-HCV II Cal1 only) and HIV.

The testing methods use assays that have been approved or cleared by the FDA or that are in compliance with the legal rules of the European Union (IVDR 2017/746/EU, IVDD 98/79/EC, Annex II, List A.)

The serum containing anti-HCV (A-HCV II 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.[8,9]

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 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.

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.

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 first opening at 2-8 °C	8 weeks
on the analyzers	31 days if continuously stored onboard (20-25 °C) or 7 weeks and up to 80 hours in total onboard (20-25 °C) if stored alternately in the refrigerator and on the analyzer

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** 411 analyzer at 20-25 °C	up to 5 hours
on the **cobas e** 601 and **cobas e** 602 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 in a sufficient number and found acceptable.

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

Li-, Na-heparin, K_2-EDTA, K_3-EDTA, plasma gel separation, and sodium citrate plasma.

Stable for 21 days at 2-8 °C, 3 days at 25 °C, 3 months at - 20 °C (± 5 °C). Freeze no more than 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.

Specimens should not be subsequently altered with additives (e.g. biocides, anti-oxidants or substances that could possibly change the pH or ionic strength of the sample) in order to avoid erroneous findings.

Centrifuge samples containing precipitates 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.

Sample stability claims were established by experimental data by the manufacturer or based on reference literature and only for the temperatures/time frames as stated in the Method Sheet. It is the responsibility of the individual laboratory to use all available references and/or its own studies to determine specific stability criteria for its laboratory.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 03290379190, PreciControl Anti-HCV, for 16 x 1.3 mL
- General laboratory equipment
- **cobas e** analyzer

Additional materials 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

Additional materials for **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 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- 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

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Additional materials for all analyzers:

- REF 11298500160, 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.

cobas e 601 and **cobas e** 602 analyzers: PreClean M solution is necessary.

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.

Ensure the calibrators are at 20-25 °C prior to measurement.

After calibration has been performed, store the calibrators at 2-8 °C, or discard.

Calibration

No internationally accepted standard for anti-HCV exists.

Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet.

Calibration frequency: Calibration must be performed once per reagent lot using A-HCV II Cal1, A-HCV II 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 4 weeks 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 outside the defined limits

Quality control

For quality control, use PreciControl Anti-HCV.

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.

The recommended quality control material is serum based. The user is responsible for providing alternate control material for plasma samples when necessary.

Note:
For technical reasons re-assigned target values and ranges valid only for a specific reagent and control lot combination must be entered manually on all analyzers. Therefore, always refer to the respective value sheet 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.

Calculation

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

The result of a sample is given in the form of a cutoff-index (signal sample/cutoff) with a result interpretation of:

- "non-reactive" (COI[c)] < 0.90)
- "border"[d)] (0.90 ≤ COI < 1.00) or
- "reactive" (COI ≥ 1.00)

c) COI = cutoff index

d) border = borderline

Interpretation of the results

Initial Elecsys Anti-HCV II assay			
COI	Result	Interpretation of results	Retest procedure
< 0.90	Non-reactive[e)]	No antibodies to HCV were detected	No retest required
0.90 ≤ COI < 1.00	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys Anti-HCV II assay
≥ 1.00	Reactive	Antibodies to HCV detected	Presumptive HCV infection, follow CDC recommendations for supplemental testing

e) Please note, per www.CDC.gov: If a patient is known to be at high risk of HCV infection, or is symptomatic, and the physician's suspicion of HCV infection is high, HCV RNA testing is often employed and is of diagnostic value, even after an initial negative anti-HCV test result.

Final Elecsys Anti-HCV II assay			
Initial result	Result after retest (COI)	Final results	Interpretation of results
Non-reactive	No retest required	NON-REACTIVE[e)]	Antibodies to HCV were not detected; does not exclude the possibility of exposure to HCV
Border	If 2 of the 3 results have a COI < 1.00	NON-REACTIVE	Antibodies to HCV were not detected; does not exclude the possibility of exposure to HCV
	If 2 of the 3 results have a COI ≥ 1.00	REACTIVE	Presumptive evidence of antibodies to HCV. Follow CDC recommendations for supplemental testing.
Reactive	No retest required	REACTIVE	Presumptive evidence of antibodies to HCV. Follow CDC recommendations for supplemental testing.

Cutoff determination

The cutoff value was established with in-house studies by measuring a panel of 1336 samples.

A Receiver Operator Curve (ROC) analysis was used to optimize sensitivity and specificity.

Validation of the cutoff was performed by external clinical studies.

Limitations

Current methods for the detection of antibodies to HCV may not detect all infected individuals. A non-reactive test result does not exclude the possibility of exposure to HCV.

Samples with visible signs of hemolysis should be checked for hemoglobin concentration before being analyzed with the Elecsys Anti-HCV II assay and should not be used if the hemoglobin concentration is greater than the level indicated in the Endogenous interference section. If necessary, a new sample should be obtained and tested.

Sample stability studies were performed using serum only.

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Drug interference studies were performed in vitro, and may not assess the potential interferences that might be seen after the drugs are metabolized in-vivo.

A reactive anti-HCV result does not exclude co-infection by another hepatitis virus.

Negative anti-HCV test results may occur during early infection due to delayed seroconversion.

The detection of anti-HCV antibodies indicates a present or past infection with hepatitis C virus, but does not differentiate between acute, chronic or resolved infection.

False positive results due to non-specific reactivity cannot be ruled out with the Elecsys Anti-HCV II assay.

False negative results may occur due to antibody levels below the detection limit of this assay or if the patient´s antibodies do not react with the antigens used in this test.

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.

Results obtained with the Elecsys Anti-HCV II assay may not be used interchangeably with values obtained with different manufacturers' assay methods.

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

Within-laboratory precision

Within-laboratory precision was determined on the **cobas e** 601 analyzer using 1 lot of Elecsys reagent to test 5 serum pools and 2 controls according to the CLSI (Clinical and Laboratory Standards Institute) guideline EP5-A3; 2 runs per day in duplicate each for 12 days (n = 48). Results are presented in the following table:

cobas e 601 analyzer					
		Repeatability[f]		**Within-laboratory**	
Sample	Mean COI	SD[g] COI	CV %	SD COI	CV %
Human serum, negative	0.038	0.001	2.5	0.001	2.5
Human serum, high negative	0.776	0.026	3.4	0.034	4.3
Human serum, low positive	1.06	0.013	1.2	0.020	1.8
Human serum, positive	1.99	0.035	1.8	0.048	2.4
Human serum, positive	5.65	0.085	1.5	0.113	2.0
PreciControl Anti-HCV1	0.048	0.001	1.9	0.001	1.9
PreciControl Anti-HCV2	3.34	0.043	1.3	0.069	2.1

f) Repeatability = within-run precision

g) SD = standard deviation

Reproducibility

Precision was further evaluated incorporating between-run, between-day, between-lot and between-site variation on the **cobas e** 601 analyzer. A reproducibility study was conducted following CLSI EP5-A2 and CLSI EP15-A2 at 3 sites incorporating a 7 member panel consisting of 5 serum pools (high negative, low positive and moderately positive) and 2 controls that were assayed for 5 days, 2 runs per day, 3 replicates per run. The analysis of data was based on guidance from CLSI documents EP5-A2 and EP15-A2. Data from all 3 reagent lots were combined to achieve SD and percent CV for repeatability (within-run), between-run, between-day, between-lot, between-site and reproducibility. The overall reproducibility (imprecision) data are summarized in the following tables:

Elecsys Anti-HCV II system reproducibility on the **cobas e** 601 analyzer					
Sample		HS07[h]	HS02[i]	HS01[j]	HS04[j]
N		180	180	180	180
Mean	COI	0.730	1.034	1.037	1.330
Repeatability	SD	0.008	0.010	0.014	0.014
	CV %	1.1	1.0	1.3	1.1
Between-run[j]	SD	0.013	0.019	0.016	0.025
	CV %	1.8	1.9	1.5	1.9
Between-day	SD	0.010	0.000[k]	0.013	0.010
	CV %	1.3	0.0	1.2	0.8
Between-lot	SD	0.068	0.109	0.048	0.138
	CV %	9.3	10.6	4.6	10.4
Between-site	SD	0.038	0.047	0.077	0.056
	CV %	5.2	4.5	7.4	4.2
Reproducibility	SD	0.080	0.121	0.094	0.153
	CV %	10.9	11.7	9.0	11.5

h) Human serum, high negative

i) Human serum, low positive

j) Between-run = intermediate precision

k) SD of 0 due to variance contributed by particular component was below stated significant figure

Elecsys Anti-HCV II system reproducibility on the **cobas e** 601 analyzer				
Sample		HS06[l]	PC[m]A-HCV1	PC A-HCV2
N		180	180	180
Mean	COI	2.660	0.056	4.025
Repeatability	SD	0.032	0.001	0.075
	CV %	1.2	2.6	1.9
Between-run	SD	0.040	0.001	0.067
	CV %	1.5	1.0	1.7
Between-day	SD	0.041	0.000[k]	0.152
	CV %	1.6	0.7	3.8
Between-lot	SD	0.111	0.002	0.356
	CV %	4.2	4.4	8.8
Between-site	SD	0.193	0.000[o]	0.271
	CV %	7.3	0.0	6.7
Reproducibility	SD	0.232	0.003	0.483
	CV %	8.7	5.2	12.0

l) Human serum, positive

m) PreciControl

Endogenous interferences

To evaluate the effect of elevated levels of hemoglobin, bilirubin, and intralipid on the Elecsys Anti-HCV II assay, 1 negative, 1 high negative, 1 low positive, and 1 positive anti-HCV sample were spiked with potential interferents. Each interferent was evaluated at 10 concentrations. All samples were tested in duplicate.

For biotin, serum samples that contain biotin at a concentration of 1200 ng/mL demonstrate ≤ 10 % bias in COI values. Pharmacokinetic studies have shown that serum concentrations of biotin can reach up to 355 ng/mL within the first hour after biotin ingestion for subjects consuming supplements of 20 mg biotin per day[10] and up to 1160 ng/mL for subjects after a single dose of 300 mg biotin.[11]

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The results of the interferences are presented in the following table:

Interferent tested	Concentration tested
Hemoglobin	≤ 0.621 mmol/L or ≤ 1.0 g/dL
Bilirubin	≤ 1129 µmol/L or ≤ 66 mg/dL
Lipemia	≤ 2000 mg/dL
Biotin	≤ 4912 nmol/mL or ≤ 1200 ng/mL
Rheumatoid factors	≤ 1200 IU/mL
Albumin	≤ 7 g/dL
IgG	≤ 7 g/dL
IgA	≤ 1.6 g/dL
IgM	≤ 1g/dL

Drug interferences

A drug interference study was performed with 17 common therapeutic drugs and 3 special therapeutic drugs used as antiviral therapeutics in chronic hepatitis C treatments. Each drug was tested 5-fold spiked into a negative, a low positive, a high negative and a positive sample. Each drug was found to be non-interfering at the following claimed concentrations:

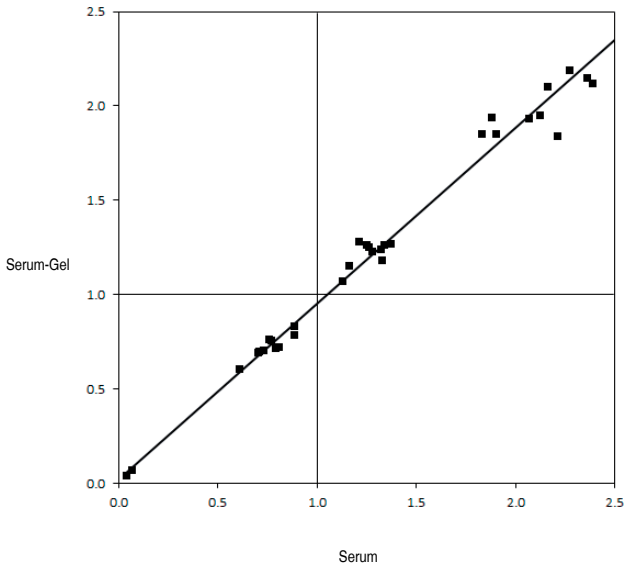
Compound	Concentration
Acetylcysteine	150 mg/L
Ampicillin-Na	75 mg/L
Ascorbic acid	52.5 mg/L
Cyclosporine	1.8 mg/L
Cefoxitin	750 mg/L
Heparin	3300 U/L
Itraconazole	30 mg/L
Levodopa	7.5 mg/L
Methyldopa+ 1.5	22.5 mg/L
Metronidazole	123 mg/L
Phenylbutazone	321 mg/L
Doxycycline	18 mg/L
Acetylsalicylic acid	30 mg/L
Rifampicin	48 mg/L
Acetaminophen	156 mg/L
Ibuprofen	219 mg/L
Theophylline	60 mg/L
PEG interferon	0.18 µg/mL
Ribavirin	1200 mg/L
Interferon-alpha2α	3000 IU/mL

Matrix effects

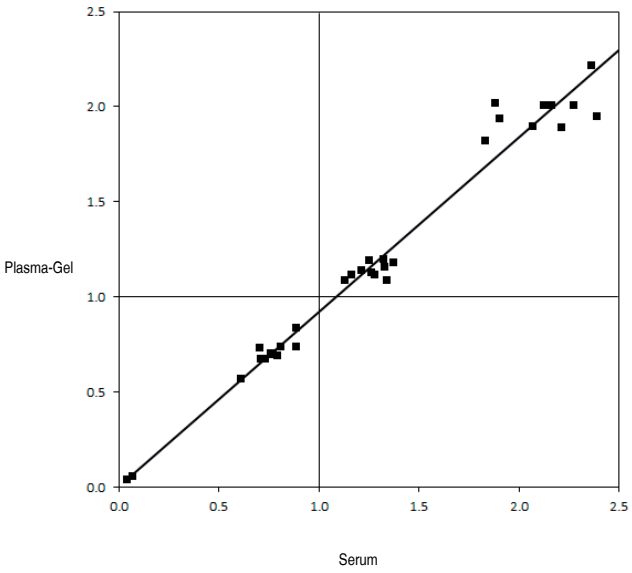
Studies were conducted to evaluate the suitability of the following 7 sample types: serum/gel separation tubes, plasma/gel separation tubes, lithium heparin plasma, K_2-EDTA plasma, K_3-EDTA plasma, sodium heparin plasma, and sodium citrate plasma to be used with the Elecsys Anti-HCV II assay. Samples were collected into matched serum and plasma collection tubes and assayed in triplicate. The study was conducted using negative, high-negative, low-positive, and positive samples for anti-HCV. The studies support the use of serum/gel separation tubes, plasma/gel separation tubes, and the following plasma types:

Lithium heparin plasma, K_2-EDTA plasma, K_3-EDTA plasma, sodium heparin plasma, and sodium citrate plasma.

Serum/Serum-Gel-Separation
y = 0.952x + 0.0011
r = 0.995



Serum/Plasma-Gel-Separation
y = 0.918x + 0.0015
r = 0.993



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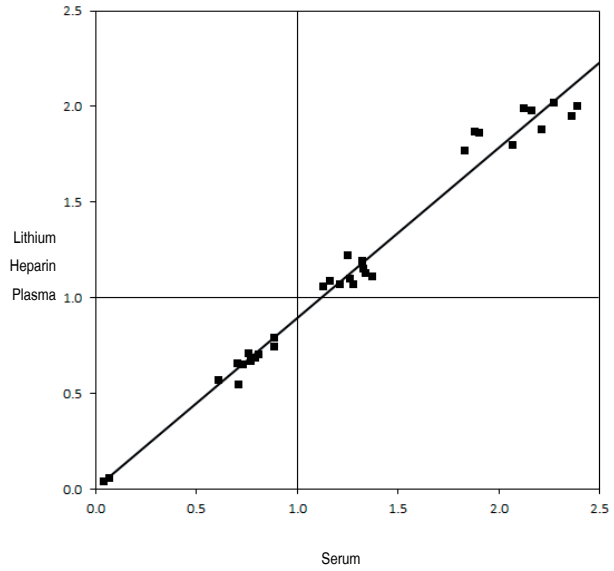
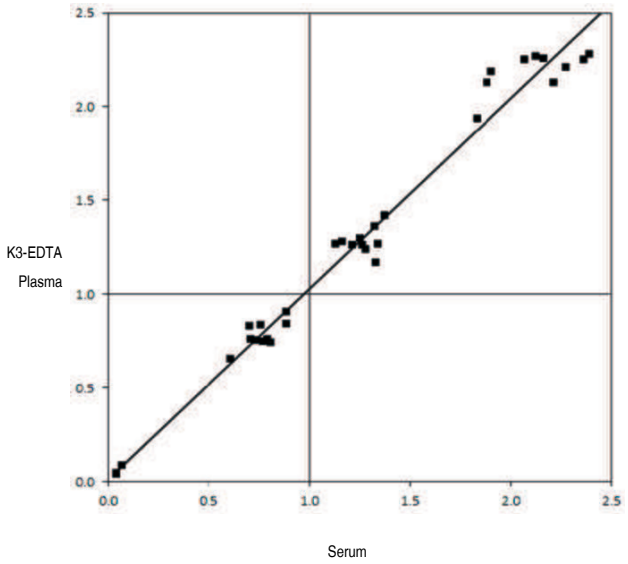
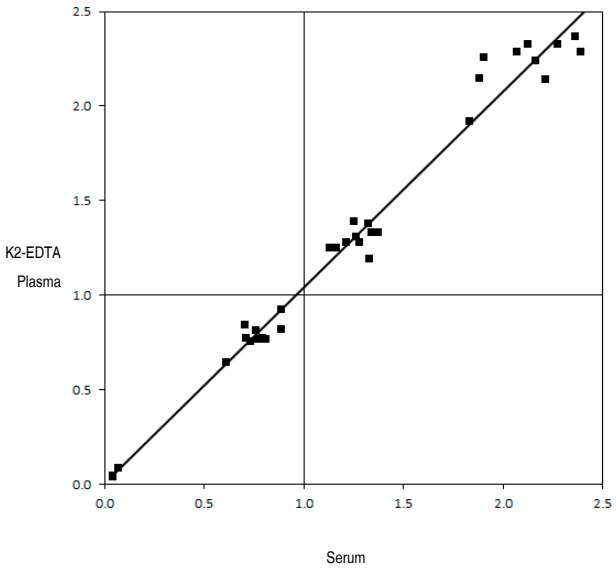
Serum/Lithium Heparin Plasma
y = 0.887x + 0.0025
r = 0.995



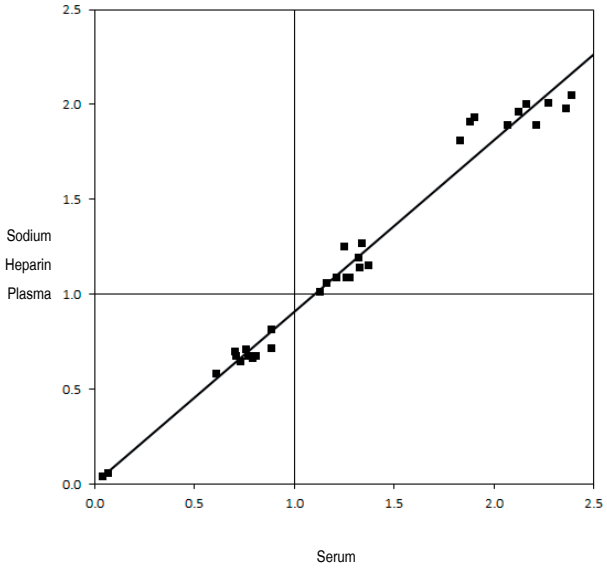
Serum/K_3-EDTA Plasma
y = 1.028x + 0.0012
r = 0.993



Serum/K_2-EDTA Plasma
y = 1.041x + 0.0012
r = 0.994



Serum/Sodium Heparin Plasma
y = 0.902x + 0.0019
r = 0.994



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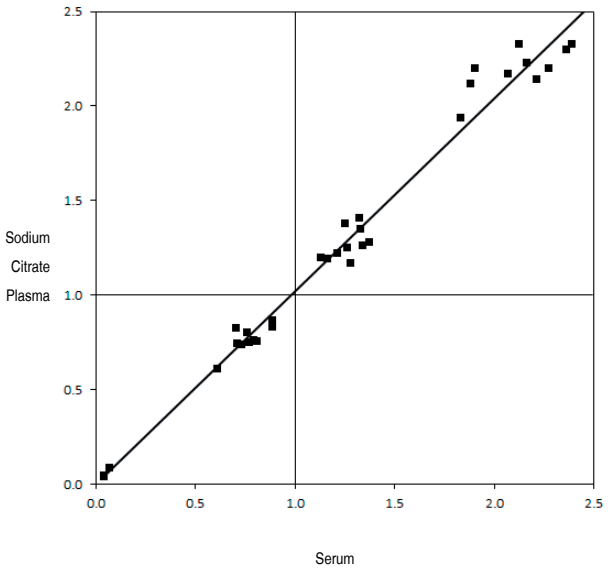
Serum/Sodium Citrate Plasma
y = 1.017x + 0.0021
r = 0.994



Analytical specificity

A study was conducted to evaluate the Elecsys Anti-HCV II assay for potential cross-reactivity in specimens from individuals with various medical conditions. All specimens in the study were evaluated with the Elecsys Anti-HCV II assay and the reference assay.

The results are summarized in the following table:

Reactivity of the Elecsys Anti-HCV II assay in individuals with various medical conditions

Category	Reference assay				Total
	Reactive		Non-reactive		
	Elecsys Anti-HCV II assay				
	RX[n)]	NR[o)]	RX	NR	
Immune disorders					
Anti-mitochondrial antibody (AMA)	3[p)]	0	0	12	15
Anti-nuclear antibody (ANA)	0	0	0	6	6
Rheumatoid factor	0	0	0	11	11
Non-viral infections					
E. coli	0	0	0	12	12
Syphilis	0	0	0	11	11
Toxoplasmosis	0	0	0	11	11
Viral infection					
Cytomegalovirus	0	0	0	12	12
Dengue fever	0	0	0	12	12
Epstein-Barr Virus	0	0	0	11	11
Hepatitis A Virus	0	0	0	10	10
Hepatitis B Virus	0	0	0	10	10
Hepatitis D Virus	8[v)]	0	0	10	18
Hepatitis E Virus	0	0	0	24	24
Human Immunodeficiency Virus	1	0	0	10	11
Herpes Simplex Virus	0	0	0	12	12
Kunjin fever	0	0	0	1	1
Murray valley / Australian encephalitis	0	0	0	4	4
Parvovirus B_{19}	0	0	0	12	12
Rubella	0	0	0	12	12
Varicella zoster	0	0	0	12	12
West Nile Virus	0	0	0	12	12
Non-viral liver disease					
Alcohol liver disease	0	0	0	5	5
Non-alcohol steatohepatitis	0	0	0	6	6
Various cirrhosis	0	0	0	7	7
Other non-viral liver disease	0	0	0	13	13
Vaccination					
HAV vaccination	0	0	0	10	10
HBV vaccination	0	0	0	10	10
Flu vaccination	0	0	0	9	9

n) RX = reactive

o) NR = non-reactive

p) These samples were not further tested because no FDA approved anti-HCV test has demonstrated adequate lack of cross-reactivity in samples with these disease states.

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HCV II assay has been shown by testing 16 commercial seroconversion panels in comparison to a reference anti-HCV assay. The comparison of the seroconversion detection between the 2 assays is summarized in the following table:

Days to evidence of anti-HCV antibody seroconversion for Elecsys Anti-HCV II assay on the **cobas e** 601 analyzer compared to the reference assay

	Reference assay		Elecsys Anti-HCV II assay		Difference in days to Elecsys Anti-HCV II reactivity (Reference-Test)[q)]
Panel ID	NR[r)]	RX[s)]	NR	RX	
6212	0	12	na[t)]	0	1 to 12
6222	26	36	26	36	0
9041	31	62	31	62	0
9044	17	21	17	21	0
9045	32	37	26	32	1 to 5
9046	0	67	0	67	0
9047	21	28	21	28	0
9058	3	7	0	3	1 to 4
PHV 911	3	14	3	14	0
PHV 913	2	7	2	7	0
PHV 914	9	12	5	9	1 to 3
PHV 917	22	85	22	85	0
PHV 918	16	24	16	24	0
PHV 921	7	14	0	4	4 to 10
PHV 922	10	14	10	14	0

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Days to evidence of anti-HCV antibody seroconversion for Elecsys Anti-HCV II assay on the **cobas e** 601 analyzer compared to the reference assay					
	Reference assay		Elecsys Anti-HCV II assay		Difference in days to Elecsys Anti-HCV II reactivity (Reference-Test)[q)]
Panel ID	NR[r)]	RX[s)]	NR	RX	
PHV 923	11	21	2	9	3 to 12

q) The dates of the first reactive test results were compared in the reference assay and Elecsys Anti-HCV II assay. If the first reactive test result occurred on the same day, then the difference is 0; if Elecsys Anti-HCV II assay had an earlier date, then the difference is positive; if Elecsys Anti-HCV II assay had a later date, then the difference is negative.

r) NR = non-reactive

s) RX = reactive

t) na = not applicable

The Elecsys Anti-HCV II assay was reactive in the same bleed as the reference assay in 10 of the 16 panels tested. The Elecsys Anti-HCV II assay was reactive earlier than the reference assay in 6 panels.

Genotype detection

The study was performed to evaluate the ability of the Elecsys Anti-HCV II assay on the **cobas e** 601 analyzer to detect antibodies to various known HCV genotypes and subtypes. 3 genotyping panels from SeraCare were available for the genotype study and consisted of the following genotypes, as determined by the specimen vendor with commercially available HCV RNA assays: 1, 2, 3, 4, 5 and 6. The panels were tested with the Elecsys Anti-HCV II assay on the **cobas e** 601 analyzer and the reference anti-HCV assay and final results were compared. The positive samples were all detected by the Elecsys Anti-HCV II assay on the **cobas e** 601 analyzer.

Summary of clinical performance

Study description

A prospective multicenter study was conducted on the **cobas e** 601 analyzer to evaluate the ability of the Elecsys Anti-HCV II assay to detect anti-HCV antibodies in specimens from an intended use diagnostic population.

2435 specimens were obtained from individuals at increased risk of HCV infection due to lifestyle, behavior, occupation, disease state or known exposure event, or from individuals with signs and symptoms of a hepatitis infection. They included 192 pediatric specimens ages 2-21 and 205 specimens from pregnant women.

The specimens were prospectively collected from 7 collection sites located in Miami, FL (171, 7.0 %), Los Angeles, CA (735, 30.2 %), Industry, CA (437, 17.9 %), San Antonio, TX (447, 18.4 %), Minneapolis, MN (156, 6.4 %), Baltimore, MD (328, 13.5 %) and Darby, PA (161, 6.6 %). Testing of the specimens was performed at 4 clinical testing sites located in St. Louis, MO, Miami, FL, South Bend, IN and Louisville, KY.

Demographic summary of overall specimen population by race		
	Adult and pediatric	
Race	N	%
American Indian / Alaska Native	20	0.82
Asian	22	0.90
African American / Black	1054	43.3
Caucasian / White	1278	52.5
Pacific Islander	6	0.25
Unknown	11	0.45
Other	44	1.84
Total	2435	100

Results by specimen classification

Following testing using the reference anti-HCV assay and the supplemental assays, the 2435 specimens were assigned an HCV status of **HCV Infected, Not Determined** or **Not HCV Infected** based on the HCV status algorithm provided in the following table:

HCV status algorithm					
Reference assay	Comp[u)] assay #1	Comp assay #2	Inter-mediate HCV status	COBAS AMPLICOR Hepatitis Virus test, Ver 2.0	HCV infection status
Reactive	-	-	nd[v)]	Negative	Not HCV infected[w)]
Reactive	+ or EQ[x)]	-	nd	Negative	nd
	- or EQ	+			
Reactive	+ or EQ	-	nd	Positive	HCV infected
	- or EQ	+			
	-	-			
Negative	Not applicable		Not HCV infected	Not applicable	Not HCV infected[w)]
Reactive	+	+	HCV infected	Not applicable	HCV infected

u) Comp = Comparator

v) nd = not determined

w) Negative test result does not exclude the possibility of exposure to hepatitis C virus.

x) EQ = equivocal

Note: Equivocal comparator assay #1 results lead to an Intermediate HCV status of "Not determined".

Comparison of results

The Elecsys Anti-HCV II assay results were compared to HCV status according to a ranking of the risk of HCV infection. The risk of HCV infection was ranked based on a clinical evaluation of the likelihood of acquiring HCV through each mode of transmission. The mode of transmission was ranked higher if the likelihood of acquiring HCV was greater. Each specimen was assigned only 1 risk (highest ranked risk). Of the 2243 at risk adult specimens analyzed, the status of 557 was **HCV Infected**. The status of 1657 specimens was **Not HCV Infected**. 29 specimens had the status **Not Determined**. The comparison of Elecsys Anti-HCV II results by HCV status is presented in the following table:

Comparison of Elecsys Anti-HCV II results to the intermediate HCV status for the adult increased risk population							
Hepatitis rank risk	Intermediate HCV status						Total
	HCV infected		Not determined		Not HCV infected		
	Elecsys Anti-HCV II result						
	RX	NR	RX	NR	RX	NR	
Signs and symptoms	241	0	7	3	1	470	721
Clotting factor recipients	1	0	0	0	0	3	4
User of IV drugs	173	0	4	1	0	82	260
Dialysis	2	0	0	0	0	3	5
Transfusion/ transplant	6	0	0	0	0	21	27
High risk sex	70	0	4	5	1	684	764
Healthcare worker	4	0	0	0	1	89	94
Other risks	61	0	3	2	0	302	368
Total	557	0	18	11	3	1654	2243

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The results of 29 samples with **Not Determined** status were subjected to HCV RNA testing. The results are presented in the following table:

Hepatitis ranked risk group	Samples (n)	HCV RNA result	Elecsys Anti-HCV II result	Final HCV infection status
Signs and symptoms	2	Negative	Non-reactive	Not HCV infected
	1	Negative	Non-reactive	Not determined
	4	Negative	Reactive	Not HCV infected
	3	Negative	Reactive	Not determined
User of IV drugs	1	Negative	Non-reactive	Not HCV infected
	3	Negative	Reactive	Not HCV infected
	1	Negative	Reactive	Not determined
High risk sex	4	Negative	Non-reactive	Not HCV infected
	1	Negative	Non-reactive	Not determined
	3	Negative	Reactive	Not HCV infected
	1	Positive	Reactive	HCV infected
Other risks	2	Negative	Non-reactive	Not HCV infected
	2	Negative	Reactive	Not HCV infected
	1	Negative	Reactive	Not determined
Total	29			

The Elecsys Anti-HCV II assay result compared to the final infection status for the adult at risk population is given in the following table:

Elecsys Anti-HCV II results on the cobas e 601 analyzer versus final HCV infection status for the adult at increased risk for hepatitis cohort

Elecsys Anti-HCV II result	Final HCV infection status			Total
	HCV infected	Not determined	Not HCV infected	
Reactive	558	5	15	578
Non-reactive	0	2	1663	1665
Total	558	7	1678	2243

Percent agreement

The positive percent agreement (PPA) and negative percent agreement (NPA) between the Elecsys Anti-HCV II assay result and the HCV status, and their corresponding 95 % confidence intervals were calculated for the study population. The results for the adult at risk population stratified by hepatitis risk group are presented in the following table:

Elecsys Anti-HCV II results versus HCV status percent agreement among study subjects ranked according to risk for HCV infection

Ranked risk	PPA % (x/n)	95 % exact confidence interval	NPA % (x/n)	95 % exact confidence interval
Signs and symptoms	99.6 (240/241)	97.7-99.99	98.3 (472/480)	96.7-99.3
Recipients of clotting factor	100 (1/1)	2.50-100	100 (3/3)	29.2-100
User of IV drugs	100 (173/173)	97.9-100	95.4 (83/87)	88.6-98.7
Dialysis	100 (2/2)	15.8-100	100 (3/3)	29.2-100
Transfusion/ transplant	100 (6/6)	54.1-100	100 (21/21)	83.9-100

Elecsys Anti-HCV II results versus HCV status percent agreement among study subjects ranked according to risk for HCV infection

Ranked risk	PPA % (x/n)	95 % exact confidence interval	NPA % (x/n)	95 % exact confidence interval
High risk sex	98.6 (71/72)	92.5-99.96	99.4 (688/692)	98.5-99.8
Healthcare worker	100 (4/4)	39.8-100	98.9 (89/90)	94.0-99.97
Other risks	100 (61/61)	94.1-100	99.0 (304/307)	97.2-99.8
Total	99.6 (558/560)	98.7-99.96	98.8 (1663/1683)	98.2-99.3

The positive percent agreement between the Elecsys Anti-HCV II assay results and the **HCV Infected** status for the adult at-risk population (n = 2243) base was 99.6 % (558/560) with a 95 % confidence interval of 98.7 to 99.96 %. The negative percent agreement between the Elecsys Anti-HCV II assay results and the **Not HCV Infected** status was 98.8 % (1663/1683) with a 95 % confidence interval of 98.2 to 99.3 %.

Elecsys Anti-HCV II results versus HCV infection status percent agreement among pregnant study subjects

	Final HCV infection status		
	Total		
Elecsys Anti-HCV II result	Infected	Not determined	Not infected
Reactive	1	0	1
Non-reactive	0	0	203
Total	1	0	204

The positive percent agreement between the Elecsys Anti-HCV II assay results and the **HCV infected** status for the pregnant population (n = 205) base was 100 % (1/1) with a 95 % confidence interval of 2.50 to 100 %. The negative percent agreement between the Elecsys Anti-HCV II assay results and the **Not HCV infected** status was 99.5 % (203/204) with a 95 % confidence interval of 97.3 to 99.99 %.

Elecsys Anti-HCV II results versus HCV infection status percent agreement among pediatric study subjects

	Final HCV infection status		
	Total		
Elecsys Anti-HCV II result	Infected	Not determined	Not infected
Reactive	2	0	3
Non-reactive	0	0	187
Total	2	0	190

The positive percent agreement between the Elecsys Anti-HCV II assay results and the **HCV Infected** status for the pediatric population (n = 192) base was 100 % (2/2) with a 95 % confidence interval of 15.8 to 100 %. The negative percent agreement between the Elecsys Anti-HCV II assay results and the **Not HCV Infected** status was 98.4 % (187/190) with a 95 % confidence interval of 95.5 to 99.7 %.

Pediatric vs adult comparison (analytical)

A study was conducted to evaluate the results observed when pediatric samples are tested with the Elecsys Anti-HCV II assay. A total of 31 pediatric (ages 2-20 years) and 31 adult serum samples were spiked with anti-HCV positive stock to yield samples at the following analyte levels: negative (5 samples), close-to-cutoff (6 samples) and positive (20 samples). All samples were tested in triplicate before and after spiking. Based on the spike level, the positive interpretation of the samples remained the same between adults and pediatrics.

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The distribution of percent bias (±) between the index values of the spiked pediatric serum samples and the mean index values of the adult serum samples are summarized in the following table:

Adult spiked observed mean (COI)	Number tested (n)	Distribution of % bias		
		X < 10 %	10 % ≤ X ≤ 20 %	X > 20 %
Negative (< 0.8)	5	20.0 (1/5)	60.0 (3/5)	20.0 (1/5)
Close-to-cutoff (0.8-1.0)	6	16.7 (1/6)	83.3 (5/6)	0.0 (0/6)
Positive (2.0-4.0)	20	15.0 (3/20)	85.0 (17/20)	0.0 (0/20)
Total	31	16.0 (5/31)	81.0 (25/31)	3.0 (1/31)

Expected results (at risk population)

The 2435 specimens from subjects at risk of HCV infection were collected from 7 collection sites in the US. A demographic summary of the at risk subjects by race/ethnic group is provided in the following table:

Demographic summary of at risk population by race		
	Adult and pediatric	
Race	N	%
American Indian / Alaska Native	20	0.82
Asian	22	0.90
African American / Black	1054	43.3
Caucasian / White	1278	52.5
Pacific Islander	6	0.25
Unknown	11	0.45
Other	6	0.25
Multiracial	38	1.56
Total	2435	100

Of the 2435 at risk subjects, 1247 (51.2 %) were female and 1188 (48.8 %) were male. The mean age was 41.8 years (age range: 2 to 84 years).

The Elecsys Anti-HCV II assay was reactive in 583 (23.9 %) of the individuals in the at risk population. Testing of the specimens was performed at 4 clinical testing sites located in St. Louis, MO, Miami, FL, South Bend, IN and Louisville, KY.

The distribution of Elecsys Anti-HCV II **Reactive** and **Non-reactive** results by age range and gender is presented in the following table:

Elecsys Anti-HCV II results by age range and gender for individuals at risk of HCV infection				
Age range (years)	Gender	Elecsys Anti-HCV II results		
		Reactive N (%)	Non-reactive N (%)	Total
2 - 11	Female	1 (6.7)	14 (93.3)	15
	Male	2 (8.0)	23 (92.0)	25
12 - 20	Female	1 (1.5)	68 (98.6)	69
	Male	0 (0.00)	35 (100)	35
21 - 29	Female	7 (2.3)	293 (97.7)	300
	Male	8 (6.2)	121 (93.8)	129
30 - 39	Female	29 (11.7)	218 (88.3)	247
	Male	34 (19.5)	140 (80.5)	174
40 - 49	Female	59 (21.6)	214 (78.4)	273
	Male	81 (24.3)	252 (75.7)	333
50 - 59	Female	102 (40.5)	150 (59.5)	252
	Male	158 (42.5)	214 (57.5)	372
60 - 69	Female	37 (47.4)	41 (52.6)	78
	Male	59 (52.2)	54 (47.8)	113
70 - 79	Female	3 (30.0)	7 (70.0)	10
	Male	2 (28.6)	5 (71.4)	7
> 80	Female	0 (0.00)	3 (100)	3
	Male	0 (0.00)	0 (0.00)	0
Totals	Female	239 (19.2)	1008 (80.8)	1247
	Male	344 (28.9)	844 (71.0)	1188
All	All	583 (23.9)	1852 (76.1)	2435

Potential cross-reactivity with HBV-infected individuals

Samples of the prospectively collected non-pregnant adult at risk cohort (2082) were tested for Hepatitis B infection (HBV). HBV-positive samples (n = 43) were identified in 2082 tested samples. Hepatitis B infection was determined by commercially available FDA-approved HBsAg and HBsAg Confirmatory assays.

The negative percent agreement between the reference methods in HBsAg-positive patients was 100 % (37/37); the positive percent agreement was 100 % (6/6).

Additional method comparison study

An additional method comparison study with clinical samples was performed comparing the Elecsys Anti-HCV II assay (REF 08837031190, biotin-updated assay) to the Elecsys Anti-HCV II assay (REF 06427405160) with a total of 219 serum samples. Of these samples, 109 were positive, 91 were negative and 19 were borderline/retest samples. The positive percent agreement was 96.33 % (90.94 % to 98.56 %)[y)] and the negative percent agreement was 99.63 % (95.27 % to 100 %)[y)]. The performance characteristics were considered equivalent.

y) 95 % CI are based on the Wilson score method, which uses an independent results assumption. This CI may be overstated.

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

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.

Symbols

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

CONTENT	Contents of kit
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REAGENT	Reagent
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→	Volume for reconstitution
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For USA: Rx only

Roche Diagnostics GmbH, Sandhofer Strasse 116, D-68305 Mannheim
www.roche.com
+800 5505 6606

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