

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

REF 03290352 160

100 tests

- Indicates analyzers on which the kit can be used

MODULAR ANALYTICS E170

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English

Warning

- Federal law restricts this device to sale and distribution by or on the order of a physician, or to a clinical laboratory; and use is restricted by or on the order of a physician.
- Assay performance characteristics have not been established in patients under the age of 21, pregnant women, or in populations of immunocompromised or immunosuppressed patients.
- This assay has not been FDA licensed for the screening of blood, plasma and tissue donors.

Intended use

The Elecsys Anti-HCV assay is an *in vitro* diagnostic test for the qualitative detection of total antibodies to hepatitis C virus (anti-HCV) in human serum or plasma (potassium EDTA, lithium heparin and sodium heparin). 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 electrochemiluminescence immunoassay "ECLIA" is intended for use on the MODULAR ANALYTICS E170 immunoassay analyzer.

Summary

Hepatitis C virus, first identified in 1989,¹ is the most common cause of posttransfusion and community-acquired non-A, non-B hepatitis worldwide. Infection with HCV frequently leads to chronic hepatitis and cirrhosis, and is associated with the development of hepatocellular carcinoma.² Common extrahepatic manifestations comprise mixed cryoglobulinemia and rheumatic diseases.³

Hepatitis C virus is an enveloped, positive-sense single-stranded RNA virus which has been classified as a genus in the family of Flaviviridae. The genome consists of ~9.5 kb encoding for a 3000 amino acid polypeptide of structural and non-structural domains.⁴ Like other RNA viruses, the HCV genome exhibits substantial heterogeneity as a result of mutations that occur during viral replication. Worldwide, at least 11 genetically distinct genotypes and multiple subtypes and virus variants have been described.⁵ Infection with specific genotypes can affect disease severity and treatment response.^{6,7} Hepatitis C is primarily transmitted through contaminated blood and blood products and to a lower extent by human body secretions.⁸

The Elecsys Anti-HCV assay is a third-generation test.^{9,10} The assay uses peptides and recombinant antigens representing core, NS3 and NS4 proteins for the determination of anti-HCV antibodies. Anti-HCV antibody tests are used in combination with other tests (e.g. HCV-RNA) to detect an infection with hepatitis C virus.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 40 µL of sample, 60 µL of a reagent containing biotinylated HCV antigens and 60 µL of a reagent containing HCV 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. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

- Results are determined automatically by the Elecsys software by comparing the electrochemiluminescence signal obtained from the sample with the cutoff value obtained by anti-HCV calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex (Ru(bpy)₃²⁺)

Reagents - working solutions

- M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
- R1 Buffer (gray cap), 1 bottle, 7 mL: HEPES^b buffer, pH 5.0.
- R2 Buffer (black cap), 1 bottle, 7 mL: HEPES buffer, pH 5.0.
- R1a Lyophilized HCV antigens, biotinylated (gray cap), 1 bottle for 1.2 mL solution.
- R2a Lyophilized HCV antigens, ruthenylated (black cap), 1 bottle for 1.2 mL solution.
- R1b Reconstitution medium for bottle R1a (gray cap), 1 bottle, 1.4 mL: Water, preservative.
- R2b Reconstitution medium for bottle R2a (black cap), 1 bottle, 1.4 mL: Water, preservative.
- Cal1 Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each: Human serum, preservative.
- 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.
- b) HEPES = [4-(2-hydroxyethyl)-piperazinol]-ethane sulfonic acid

Precautions and warnings

For *in vitro* diagnostic use.

Exercise the usual precautions required for handling all laboratory reagents.

Disposal of all waste material should be in accordance with local guidelines.

Safety data sheets available to a professional user on request.

Consider any materials of human origin as infectious and handle them using typical biosafety procedures and Universal Precautions according to the OSHA standard on Bloodborne Pathogens, 29 CFR 1910.1030.¹¹

All products derived from human blood are prepared exclusively from the blood of donors tested individually by FDA-licensed or approved tests and shown to be non-reactive for HBsAg, antibodies to HIV and antibodies to HCV (Cal1 only). The serum containing anti-HCV (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 treated as potentially infectious. In the event of exposure the directives of the responsible health authorities should be followed.^{11,12}

The reagents may not be used after the stated expiration date.

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Avoid any sample cross-contamination during sample preparation.

Reagent handling

The reagents R1 and R2 are not ready for use and have to be prepared. See "Preparation of working solutions" section for further instructions. The reagents M, Cal1 and Cal2 are ready for use and are supplied 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 for later use at 2-8 °C. Perform **only one** calibration procedure per aliquot.

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

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All information required for correct operation is read in via the respective reagent barcodes.

Preparation of working solutions

The reagents R1 and R2 are not ready for use and have to be prepared by adding the reconstituted antigens.

Note: Incubate the reconstituted reagent for at least 12 hours at 2-8 °C to finalize the reconstitution process. It is recommended to carry out this storage overnight (e.g. 16 hours at 2-8 °C).

For the reconstitution of the lyophilized antigens proceed as follows:

Using adapters

- 1a Connect bottle R1a (lyophilized biotinylated antigens; gray cap) with bottle R1b (reconstitution medium for bottle R1a; gray cap) using one of the adapters. Transfer the volume of reconstitution medium. **Avoid the formation of foam!**
- 1b Connect bottle R2a (lyophilized ruthenylated antigens; black cap) with bottle R2b (reconstitution medium for bottle R2a; black cap) using one of the adapters. Transfer the volume of reconstitution medium. **Avoid the formation of foam!**
- 2 Reconstitute the lyophilisates during 30 min ± 5 min by occasionally gently swirling until the lyophilisates are completely dissolved. **Avoid the formation of foam! Do not mix liquid back and forth into and out of the reconstitution medium bottles!**
- 3 Remove empty bottles from adapters.
- 4a Transfer the volume of the reconstituted Bi-antigen solution R1a (gray cap) into the R1 of the rackpack (gray cap).
- 4b Transfer the volume of reconstituted Ru-antigen solution R2a (black cap) into the R2 of the rackpack (black cap).
- 5 Produce homogeneous solutions (R1 and R2) by occasionally gently swirling from time to time during a time period of 15 min. **Avoid the formation of foam!**
- 6 **Incubate the reconstituted reagent for at least 12 hours at 2-8 °C to finalize reconstitution process. It is recommended to carry out this storage overnight (e.g. 16 hours at 2-8 °C).**
- 7 Reagent kit with R1 and R2 working solution is now ready for use. **Always store the kit containing the working solution R1/R2 at 2-8 °C when not in use. A stability of 14 days can only be guaranteed if R1 and R2 containing the HCV antigens are stored at 2-8 °C, and are not subjected to heat stress.**

Note: When transferring the solutions using the adapter, a volume of < 200 µL remains. This remaining volume does not need to be transferred by additional pipetting.

Storage and stability

Store at 2-8 °C. Allow all reagents to come to room temperature (20-25 °C) before use. Do not freeze the reagents.

Store the Elecsys Anti-HCV reagent kit upright in order to ensure complete availability of the microparticles during automatic mixing prior to use.

Stability:

reagent kit unopened at 2-8 °C	up to the stated expiration date
rackpack (including reconstituted antigens) at 2-8 °C	2 weeks
working reagents on MODULAR ANALYTICS E170 after opening	72 hours if continuously stored onboard (20-25 °C) or 2 weeks at 2-8 °C and up to 40 hours in total onboard (20-25 °C) if stored alternately in the refrigerator and on the analyzer

Stability of the calibrators

after opening at 2-8 °C	2 weeks
on MODULAR ANALYTICS E170 at 20-25 °C	up to 2 hours

Store calibrators upright! Ensure that no calibration solution is trapped in the opened snap-cap.

Specimen collection handling

Serum and plasma should be separated from cells within two hours from the time of collection.

Serum collected using standard sampling tubes or tubes containing separating gel, Li-heparin, Na-heparin and K₂-EDTA plasma are the recommended sample types for this assay.

Sample stability

Test samples as soon as possible after collection. Store samples at 2-8 °C if not tested immediately.

Sample stability studies were performed using serum only. Serum samples are stable for 21 days at 2-8 °C, 4 days at 25 °C, 3 months at -20 °C. Samples may be frozen and thawed up to 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 that were not tested may contain 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 patients' samples, calibrators, and controls are at ambient temperature (20-25 °C) before measurement. Because of possible evaporation effects, samples and calibrators on the analyzer should be measured within 2 hours.

Materials provided

See "Reagents - working solutions" section for reagents.

- 2 x 6 bottle labels
- 2 adapters

Materials required (but not provided)

- [REF] 03290379160, PreciControl Anti-HCV, for 8 x 1.3 mL each of PreciControl Anti-HCV 1 and 2
- General laboratory equipment
- MODULAR ANALYTICS E170 analyzer

Accessories for MODULAR ANALYTICS E170 analyzer:

- [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
- [REF] 03004899190, PreClean M, 5 x 600 mL detection cleaning solution
- [REF] 11298500160, 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 before 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.

PreClean M solution is necessary.

MODULAR ANALYTICS E170 analyzer: Bring the cooled reagents to approx. 20 °C and place on the reagent disk (20 °C) of the analyzer. **Avoid the formation of foam.** The system automatically regulates the temperature of the reagents and the opening/closing of the bottles.

Place the calibrators Cal1 and Cal2 in the sample zone of the analyzer.

Only keep open during calibration. All information necessary for calibration

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

is encoded on the barcoded bottle label and is read in automatically.
After calibration has been performed, discard.

Calibration

Calibration frequency:

Every Elecsys Anti-HCV rackpack must be calibrated using Elecsys Anti-HCV Cal1 and Cal2. **Lot calibrations are not allowed** for the Elecsys Anti-HCV assay.

Renewed calibration for each Elecsys Anti-HCV rackpack is recommended as follows:

- after 7 days (when the same reagent kit is used alternately on board the analyzer and stored refrigerated)
- as required: e.g. quality control findings outside the specified limits
- more frequently when this is required by pertinent regulations

Range for electrochemiluminescence signals (counts) for the calibrators:

Negative calibrator (Cal1): 350-1600.

Positive calibrator (Cal2): 10000-60000.

No internationally accepted standard for anti-HCV exists. Calibrator and Control materials are traceable to a Roche internal standard. This internal standard is manufactured by diluting an anti-HCV reactive human material (recalcified human plasma) in anti-HCV non-reactive human serum..

Quality control

For quality control, use Elecsys PreciControl Anti-HCV 1 and 2.

The controls 1 and 2 should be run as single determinations at least once every 24 hours when the test is in use, once per reagent kit, and after every calibration. The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. If control results are out of their specified range, test results are invalid, and all samples in the run must be retested. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

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 valid for a specific reagent and control lot combination only, must be entered manually. Therefore, always consider the value sheet included in the rackpack or PreciControl kit 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.

Results

The analyzer automatically calculates the cutoff based on the measurement of Cal1 and 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 < 0.90)
- "border"^c (0.90 ≤ COI < 1.00) or
- "reactive" (COI ≥ 1.00)

c) border = borderline

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Interpretation of the results:

Initial Elecsys Anti-HCV Assay			
COI	Result	Interpretation of results	Retest Procedure
< 0.90	Non-reactive ^d	No antibodies to HCV were detected	No Retest required
0.90 ≤ COI < 1.00	Border	Border line zone (undetermined)	Retest in duplicate with the Elecsys Anti-HCV assay.
COI ≥ 1.00	Reactive	Antibodies to HCV detected	Presumptive HCV infection, follow CDC recommendations for supplemental testing.

d) Please note: 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 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.

e) Please note: 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.

Cut-off determination

The cut-off value was established with in-house studies by measuring a panel of 682 samples.

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

Validation of the cut-off was performed by external clinical studies.

Limitations

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

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

Samples that show visible signs of hemolysis should not be analyzed with the Elecsys Anti-HCV assay, a new sample should be obtained and tested.

In patients receiving therapy with high biotin doses (i.e. > 5 mg/day), no sample should be taken until at least 8 hours after the last biotin administration.

Do not use sodium citrate plasma with the Elecsys Anti-HCV immunoassay.

Sample stability studies were performed using serum only. Plasma sample stability has not been established. The user should follow available guidelines.

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

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*.

False positive results were observed in a limited number of patients positive for HBsAg.

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 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.

False positive results due to non-specific reactivity cannot be ruled out with the Elecsys Anti-HCV assay. Studies show there is the potential for cross-reactivity from patients with antibodies to Hepatitis D Virus or Hepatitis E Virus.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur.

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

Specific performance data

Representative performance data for the Elecsys Anti-HCV immunoassay run on the MODULAR ANALYTICS E170 analyzer are given below. Results obtained by individual laboratories may differ.

Precision

Within-laboratory precision

Within-laboratory precision was determined on the MODULAR ANALYTICS E170 analyzer using one lot of Elecsys reagent to test 4 serum pools and 2 controls according to the CLSI (Clinical and Laboratory Standards Institute) guideline EP5-A2; 2 runs per day with 2 replicates each for 20 days (n = 80). Results are presented below.

Elecsys Anti-HCV Within-laboratory Precision						
Sample	n	Mean	Repeatability		Within-laboratory	
			COI ^f	SD ^g	% CV	SD
HS ^h , negative	80	0.065	0.013	20.4	0.009	14.1
HS, high negative	80	0.973	0.045	4.6	0.026	2.7
HS, low positive	80	1.04	0.047	4.6	0.023	2.3
HS, moderate positive	80	2.64	0.098	3.7	0.066	2.5
PreciControl A-HCV1	80	0.127	0.010	8.2	0.010	7.6
PreciControl A-HCV2	80	12.3	0.476	3.9	0.208	1.7

f) COI = cutoff-index

g) SD = standard deviation

h) HS = human serum

Precision was further evaluated incorporating between-run, between-day, between-lot and between-site variation. A reproducibility study was conducted following CLSI EP5-A2 and CLSI EP15-A2 at three sites incorporating a 5 member panel consisting of 3 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 three 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 imprecision data are summarized in the following table:

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Elecsys Anti-HCV system reproducibility on the MODULAR ANALYTICS E170 analyzer

Sample		HS1 ⁱ	HS2 ^j	HS3 ^k	PC 1 ^l	PC 2 ^m
N		180	180	180	180	180
Mean	COI	0.918	1.21	2.38	0.142	15.5
Repeat-ability	SD	0.024	0.031	0.052	0.030	0.372
	% CV	2.6	2.6	2.2	21.1	2.4
Between-run ⁿ	SD	0.038	0.027	0.087	0.000 ^o	0.817
	% CV	4.2	2.3	3.6	0.0	5.3
Between-day	SD	0.019	0.026	0.053	0.027	0.000 ^o
	% CV	2.1	2.2	2.2	19.3	0.0
Between-lot	SD	0.015	0.068	0.117	0.052	1.35
	% CV	1.6	5.6	4.9	36.7	8.7
Between-site	SD	0.058	0.000 ^o	0.154	0.035	0.000 ^o
	% CV	6.3	0.0	6.5	24.5	0.0
Repro-ducibility	SD	0.077	0.083	0.224	0.075	1.62
	% CV	8.4	6.9	9.4	52.6	10.5

i) Human serum high negative

j) Human serum low positive

k) Human serum moderate positive

l) PreciControl A-HCV1

m) PreciControl A-HCV2

n) between-run = intermediate precision

o) SD of zero due to variance contributed by particular component was below stated significant figure

Endogenous interferences

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

Samples containing hemoglobin at concentrations ≥ 0.1 g/dL result in reduced recovery of anti-HCV. Samples that show visible signs of hemolysis should not be analyzed with the Elecsys Anti-HCV assay, a new sample should be obtained.

The results of the other interferences are presented below:

Interferent tested:	No interference up to:
Bilirubin	50 mg/dL
Lipemia	2100 mg/dL
Biotin	50 ng/mL
Total Protein	12.0 g/dL

Drug Interferences

A drug interference study was performed with 18 common therapeutic drugs and two special therapeutic drugs used as antiviral therapeutics in chronic hepatitis C treatments. Each drug was tested three-fold spiked into a negative, a low positive and a positive sample. Each drug was found to be non-interfering at the following claimed concentrations:

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

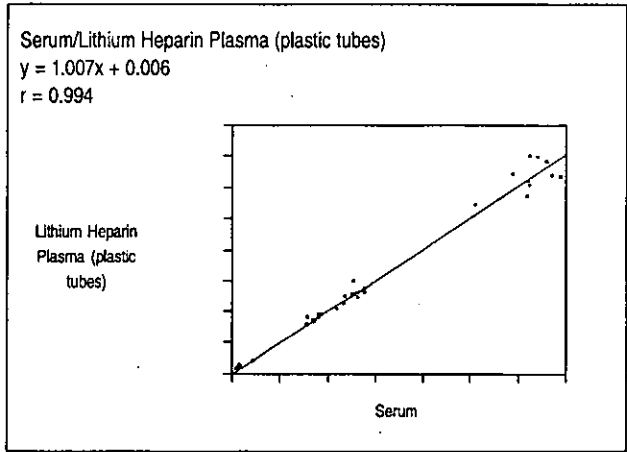
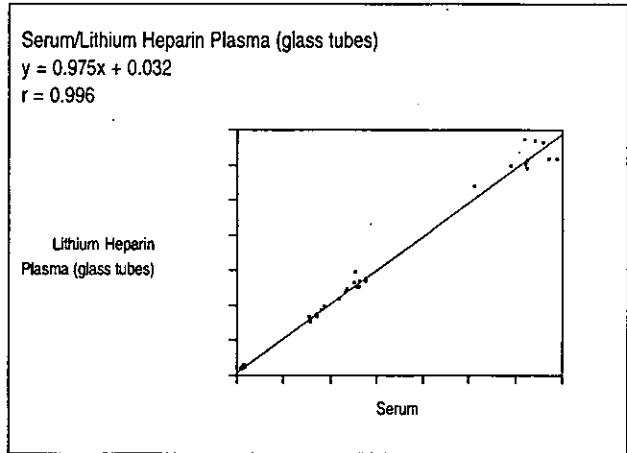
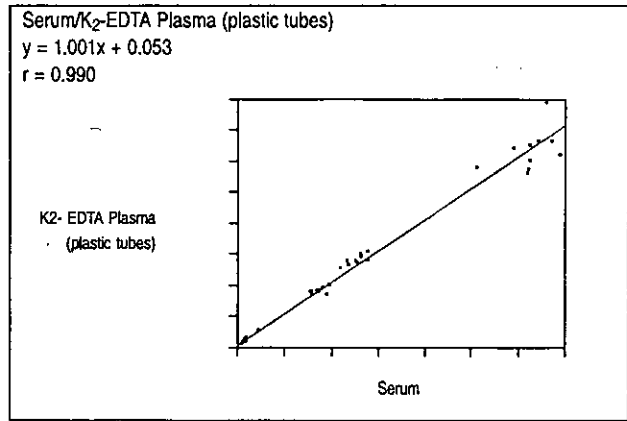
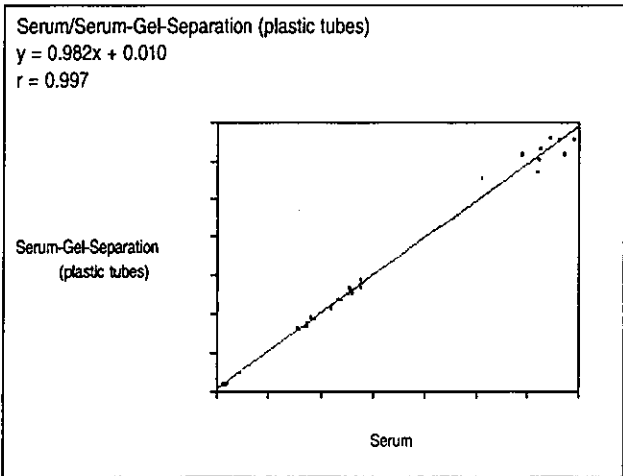


Compound	Concentration
Acetyl cysteine	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca- Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Tetracycline	50 mg/L
Acetylsalicylic acid	1000 mg/L
Rifampicin	60 mg/L
Acetaminophen	200 mg/L
Ibuprofen	500 mg/L
Theophylline	100 mg/L
Alpha-interferon	3000 U
Ribavirin	1200 mg/L

Matrix effects

Studies were conducted to evaluate the suitability of the following four types of blood collection tubes; serum/gel separation tubes, Lithium heparin plasma, K₂-EDTA plasma and sodium heparin plasma to be used with the Elecsys Anti-HCV 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 and the following plasma types:

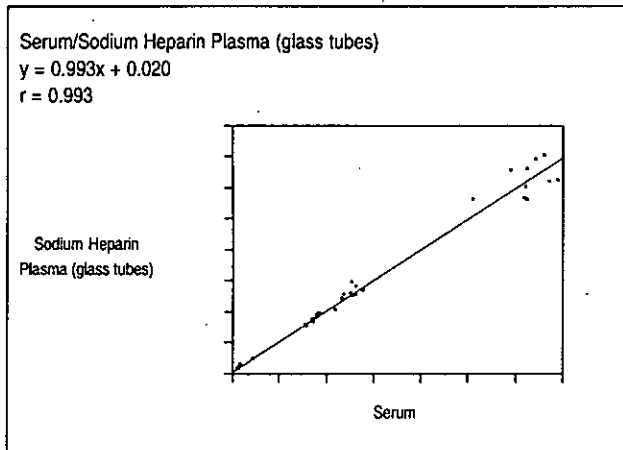
Lithium heparin plasma, K₂-EDTA plasma and sodium heparin plasma. Do not use sodium citrate plasma with the Elecsys Anti-HCV immunoassay. The results are shown below.



Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

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Analytical specificity

A study was conducted to evaluate the Elecsys Anti-HCV assay for potential cross-reactivity in specimens from individuals with medical conditions unrelated to HCV infection. All specimens in the study were found to be non-reactive (negative) in the Elecsys Anti-HCV and the reference assay. Supplemental testing was performed by RIBA testing as required. The results are summarized in the following table:

Reactivity of the Elecsys Anti-HCV assay in individuals with medical conditions unrelated to HCV infection

Category	n	Anti-HCV reference assay			
		Negative		Reactive	
		Elecsys Anti-HCV assay			
		NR ^p	RX ^q	NR	RX
Anti-nuclear antibody (ANA)	10	9	0	0	1
Cytomegalovirus (anti-CMV positive)	10	10	0	0	0
Dengue Fever	10	10	0	0	0
Elevated IgG	10	10	0	0	0
Elevated IgM	10	10	0	0	0
Elevated total bilirubin	10	8	0	1 ^r	1
Elevated total protein	10	8	0	0	2
Epstein-Barr Virus (anti-EBV positive)	10	10	0	0	0
<i>Escherichia coli</i> (E. coli)	10	8	0	0	2
HAV vaccination	10	10	0	0	0
HBV vaccination	10	10	0	0	0
Hepatitis A Virus (anti-HAV positive)	10	10	0	0	0
Hepatitis B Virus (anti-HBV positive)	10	9	0	1 ^r	0
Hepatitis D Virus (anti-HDV positive)	11	5	3 ^s	0	3
Hepatitis E Virus (anti-HEV positive)	40	4	5 ^t	1 ^r	30
Herpes Simplex Virus (HSV) IgG	10	10	0	0	0
Human immunodeficiency Virus (anti-HIV-1 positive)	10	9	0	0	1
Human T-cell Lymphotropic Virus (HTLV)	10	8	1 ^u	0	1
Influenza vaccine recipients	10	10	0	0	0
Multiparous female	10	10	0	0	0
Murray valley/Australian encephalitis	2	2	0	0	0
Non-viral liver disease	17	16	0	0	1
Parvovirus B ₁₉ infection	10	9	0	0	1
Rheumatoid Factor positive	10	9	0	0	1
Rubella	10	9	1 ^r	0	0
Syphilis (<i>T. pallidum</i>)	10	9	0	1 ^r	0
Systemic lupus erythematosus (SLE)	10	10	0	0	0
Toxoplasmosis IgG positive	10	9	0	0	1
Varicella zoster (VZV)	10	9	0	1 ^r	0
West Nile virus infection	11	11	0	0	0
Yeast infection	10	9	0	0	1

p) NR = non-reactive

q) RX = reactive

r) RIBA testing resolved results in favor of Elecsys assay

s) 2/3 RIBA testing resolved in favor of reference assay, 1 RIBA was indeterminate

t) 4/5 RIBA testing resolved in favor of reference assay, 1 RIBA was indeterminate

u) RIBA testing resolved results in favor of reference assay

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HCV assay has been shown by testing 20 commercial seroconversion panels in comparison to a reference anti-HCV immunoassay. For members of panels that had a reactive status in one assay earlier than the other assay, supplemental testing with the Chiron RIBA HCV 3.0 SIA was performed on the reactive panel members. The comparison of the seroconversion detection between the two assays is summarized in the following table:

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)

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Elecsys Anti-HCV - Days to evidence of HCV infection seroconversion panels

Panel ID	Reference anti-HCV		Elecsys Anti-HCV		Chiron RIBA HCV 3.0 SIA			Difference in days to anti-HCV reactive Reference - Elecsys ^v
	Neg ^w	RX ^x	NR ^y	RX	Neg	Ind ^z	Pos	
6216	17	23	23			23		N/A
6222	36	40	26	36	36		40	4
6224	11	19	7	11	11	19		8
6226	32	37	32	37	37	39	44	0
PHV901	65	97	65	97			97	0
PHV904	7	9	2	7	7		9	2
PHV905	14	18	7	11		11	21	7
PHV906		0		0			0	0
PHV909	0	28	30			28		N/A
PHV910	4	8	4	8			8	0
PHV911	3	14	3	14			14	0
PHV912	4	7		0	4	7		7
PHV913	2	7	2	7		7		0
PHV914	12	16	9	12	12	16	24	4
PHV915	5	12	5	12			12	0
PHV917	22	85	22	85			85	0
PHV918	16	24	16	24		24	27	0
PHV919		0		0		25	28	0
PHV920	5	7	7	13			7	-6
PHV921	0	4	7	14			4	-10

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

w) Neg = negative
x) RX = reactive
y) NR = non-reactive
z) Ind = indeterminate

The Elecsys Anti-HCV assay was reactive in the same bleed as the reference assay in 10 of the 20 panels tested. The Elecsys Anti-HCV assay was reactive earlier than the reference assay in 6 panels. The reference anti-HCV assay was reactive earlier than the Elecsys Anti-HCV assay in 2 panels. Seroconversion never occurred in either assay in 2 panels.

Genotype detection

The study was performed to evaluate the ability of the Elecsys Anti-HCV immunoassay on the MODULAR ANALYTICS E170 analyzer to detect antibodies to various known HCV genotypes and subtypes. Two genotyping panels from SeraCare/BBi 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 assay on the MODULAR ANALYTICS E170 analyzer and the reference anti-HCV assay and final results were compared. The Elecsys Anti-HCV assay on the MODULAR ANALYTICS E170 analyzer and the reference anti-HCV assay results were in 100 % agreement for the tested HCV genotypes.

Summary of clinical performance

Study description

A prospective multi-center study was conducted to evaluate the ability of the Elecsys Anti-HCV assay to detect anti-HCV antibodies in specimens from an intended use diagnostic population.

Of the 2097 specimens tested in the Elecsys Anti-HCV assay clinical study, 1283 specimens were obtained from individuals at risk of HCV infection due to lifestyle, behavior, occupation, disease state or known exposure event; and 814 specimens were obtained from individuals with signs and symptoms of a hepatitis infection.

The 2097 specimens were collected from seven collection sites located in Miami, FL (39.2 %), Los Angeles, CA (53.3 %), Newark, NJ (2.0 %) and

Atlanta, GA (5.4 %). A demographic summary of the overall specimen population by race/ethnic group is provided in the following table:

Demographic summary of overall specimen population by race		
Race	Group n	Percent %
African American / Black	1003	47.8
American Indian / Alaska Native	13	0.62
Asian	7	0.33
Caucasian / White	1054	50.3
Pacific Islander	5	0.24
Other	15	0.72
Total	2097	100

Of the 2097 at risk subjects, 609 (29.0 %) were female and 1488 (71.0 %) were male. The mean age of the subjects was 43 years (age range: 21 to 81 years). Testing of the specimens was performed at the 3 clinical testing sites located in St. Louis, MO, South Bend, IN and Fort Lauderdale, FL.

Results by specimen classification

Following testing using the reference anti-HCV assay and the supplemental assays, 2097 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 anti-HCV final test result	Chiron RIBA HCV 3.0 SIA	Indeterminate HCV status	COBAS AMPLICOR Hepatitis C Virus Test, Version 2.0	HCV status
Reactive	Indeterminate	Not determined	Negative	Not HCV infected ^{aa}
Reactive	Indeterminate	Not determined	Positive	HCV infected
Negative	Not applicable	Not HCV infected	Not applicable	Not HCV infected
Reactive	Positive	HCV infected	Not applicable	HCV infected
Reactive	Negative	Not HCV infected	Not applicable	Not HCV infected

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

Comparison of results

The Elecsys Anti-HCV 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 one risk (highest ranked risk). Of the 2097 specimens analyzed, the status of 453 was **HCV Infected**. The status of 1608 specimens was **Not HCV Infected**. 36 specimens had the status **Not Determined**. The comparison of Elecsys Anti-HCV results by HCV status is presented in the following table:

Anti-HCV

Antibody to hepatitis C virus (anti-HCV)



Comparison of Elecsys Anti-HCV Results to HCV status - for the Prospective Population by Presumptive Diagnosis and Risk Groups for HCV

Specimen population (Risk groups)	HCV status						Total
	HCV infected		Not determined		Not HCV infected		
	Elecsys Anti-HCV result						
	NR (n)	RX (n)	NR (n)	RX (n)	NR (n)	RX (n)	
Individuals at risk of HCV infection							
Signs and symptoms	0	215	2	14	570	13	814
Clotting factor recipients	0	3	0	0	8	2	13
IV drug user (current or past)	0	100	1	6	80	2	189
Dialysis	0	1	0	0	7	1	9
Transfusion /transplant	0	14	0	3	31	0	48
High risk sex	1	75	1	6	555	8	646
Healthcare worker	0	7	0	1	62	1	71
Other ranked ^{ab}	0	36	1	1	259	3	300
Other not ranked ^{ac}	0	1	0	0	5	1	7
Total	1	452	5	31	1577	31	2097

ab) Individuals with risk factors ranked lower than top 6 risks.

ac) Individuals with risk factors provided by subject that is not predefined in CRF.

The results of 36 samples with **Not Determined** status were subjected to HCV RNA testing. The results are presented in the table below:

Hepatitis risk group	Samples (N)	HCV RNA result	Elecsys Anti-HCV result	Diagnosis and interpretation
Signs and symptoms	8	Detected	RX	Anti-HCV positive
	2	Not detected	NR	Anti-HCV negative
	6	Not detected	RX	Anti-HCV negative
IV drug user (current or past)	1	Detected	RX	Anti-HCV positive
	1	Not detected	NR	Anti-HCV negative
	5	Not detected	RX	Anti-HCV negative
Transfusion / transplant	3	Not detected	RX	Anti-HCV negative
High risk sex	1	Detected	NR	Anti-HCV positive
	2	Detected	RX	Anti-HCV positive
	4	Not detected	RX	Anti-HCV negative
Healthcare worker	1	Not detected	RX	Anti-HCV negative
Other ranked ^{ad}	1	Not detected	NR	Anti-HCV negative
	1	Not detected	RX	Anti-HCV negative
Total	36			

ad) Individuals with risk factors ranked lower than top 6 risks.

Percent agreement

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

Elecsys Anti-HCV results versus HCV status percent agreement among study subjects ranked according to risk for HCV infection				
Hepatitis risk group	Positive percent agreement % (x/n)	95 % confidence interval	Negative percent agreement % (x/n)	95 % confidence interval
Signs and symptoms	100 (223/223)	98.4-100	96.8 (572/591)	95.0-98.1
Clotting factor recipients	100 (3/3)	29.2-100	80.0 (8/10)	44.4-97.5
IV drug user (current or past)	100 (101/101)	96.4-100	92.0 (81/88)	84.3-96.7
Dialysis	100 (1/1)	2.50-100	87.5 (7/8)	47.4-99.7
Transfusion /transplant	100 (14/14)	76.8-100	91.2 (31/34)	76.3-98.1
High risk sex	97.5 (77/79)	91.2-99.7	97.9 (555/567)	96.3-98.9
Healthcare worker	100 (7/7)	59.0-100	96.9 (62/64)	89.2-99.6
Other ranked ^{ae}	100 (36/36)	90.3-100	98.5 (260/264)	96.2-99.6
Other not ranked ^{af}	100 (1/1)	2.50-100	83.3 (5/6)	35.9-99.6
Total	99.6 (463/465)	98.5-99.95	96.9 (1581/1632)	95.9-97.7

ae) Individuals with risk factors ranked lower than top 6 risks.

af) Individuals with risk factors provided by subjects that is not predefined in CRF.

The positive percent agreement between the Elecsys Anti-HCV assay results and the **HCV Infected** status for the study population (n = 2097) was 99.6 % (463/465) with a 95 % confidence interval of 98.5 - 99.95 %. The negative percent agreement between the Elecsys Anti-HCV assay results and the **Not HCV Infected** status was 96.9 % (1581/1632) with a 95 % confidence interval of 95.9 - 97.7 %.

The positive percent agreement between the Elecsys Anti-HCV assay results and the **HCV Infected** status for the symptomatic study population (n=814) was 100 % (223/223) with a 95 % confidence interval of 98.4 - 100 %. The negative percent agreement between the Elecsys Anti-HCV assay results and the **Not HCV Infected** status was 96.8% (572/591) with a 95 % confidence interval of 95.0 - 98.1 %.

The positive percent agreement between the Elecsys Anti-HCV assay results and the **HCV Infected** status for the at risk population (n=1283) was 99.2% (240/242) with a 95 % confidence interval of 97.1 - 99.9 %. The negative percent agreement between the Elecsys Anti-HCV assay results and the **Not HCV Infected** status was 96.9% (1009/1041) with a 95 % confidence interval of 95.7 - 97.9 %.

Expected results (At risk population)

Of the 2097 specimens analyzed in the Elecsys Anti-HCV clinical study, a total of 1283 (61.2 %) specimens were from the individuals at risk of HCV infection. All subjects were at risk of HCV infection due to lifestyle, behavior, occupation or known exposure event but were asymptomatic and reported no current signs or symptoms of hepatitis.

The 1283 specimens from subjects at risk of HCV infection were collected at seven collection sites in the US (located in Miami, FL (52.5 %), Los Angeles, CA (36.9 %), Newark, NJ (2.6 %) and Atlanta,

PreciControl Anti-HCV (Draft)



03290379 160

16 x 1.3 mL

English

Intended use

Elecsys PreciControl Anti-HCV is used for quality control of the Elecsys Anti-HCV immunoassay on the MODULAR ANALYTICS E170 immunoassay analyzer.

Summary

Elecsys PreciControl Anti-HCV contains control serum based on human serum made from recalcified plasma in the negative and positive concentration range. The controls are used for monitoring the performance of the Elecsys Anti-HCV immunoassay.

Reagents - working solutions

- PC A-HCV1: 8 bottles, each containing 1.3 mL of control serum Human serum, negative for anti-HCV; 0.5 % Bronidox L (preservative). Target range for cutoff index: 0-0.3 COI.
- PC A-HCV2: 8 bottles, each containing 1.3 mL of control serum Anti-HCV (human) in human serum; 0.5 % Bronidox L (preservative). Target value for cutoff index: approx. 8 COI.

The exact target ranges (target value \pm 30 %), given in the form of a cutoff index, are encoded in the barcodes as well as printed on the enclosed (or electronically available) value sheet.

Value assignment and target ranges

The target values and ranges were determined and evaluated by Roche. For each lot of PreciControl Anti-HCV lot manufactured, the PreciControls are run in duplicate on at least one MODULAR ANALYTICS E170 analyzer and two Elecsys 2010 analyzers. The target value of each PreciControl is defined as the median value obtained over at least 6 determinations (duplicate runs on at least 3 analyzers) of the respective PreciControl. Traceability of the Elecsys Anti-HCV assay is given in the package insert of the respective Elecsys Anti-HCV assay. Control values have not been established for assays other than the Elecsys Anti-HCV assay. Results must be within the specified ranges. All test steps must be checked when increasing or decreasing trends or suddenly occurring deviations beyond the range limits are seen. Control values must be within the ranges specified in the electronically available value sheet. If a control result is out of its specified range, test results are invalid, and these samples must be retested. Each laboratory should establish corrective measures to be taken if values fall outside the limits.

Note:

For technical reasons re-assigned target values valid for a specific reagent and control lot combination only, must be entered manually. Therefore, always consider the value sheet included in the rackpack or PreciControl kit 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.

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 (PC A-HCV1 only) 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-HCV used for the positive control (PC A-HCV2) 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 treated just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.^{1,2}

The controls may not be used after the stated expiration date.

Avoid the formation of foam with all reagents and sample types (specimens, calibrators, and controls).

Controls are in a serum matrix made from recalcified plasma. The user should provide alternate control material for plasma when necessary. The controls are not calibrators and should not be used for assay calibration.

Handling

The controls are supplied ready-for-use in bottles compatible with the system. The controls should only be left on the analyzer during performance of quality control. After use, close the bottles as soon as possible and store upright at 2-8 °C.

One should not perform more than 7 quality control procedures per bottle to minimize the effect of potential evaporation.

Storage and stability

Store at 2-8 °C. Store controls upright in order to prevent the control solution from adhering to the snap-cap.

Stability:

unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzer at 20-25 °C	up to 5 hours

Materials provided

- Elecsys PreciControl Anti-HCV, 2 barcode cards, control barcode sheet

Materials required (but not provided)

- MODULAR ANALYTICS E170 immunoassay analyzer and assay reagents. See assay package insert and operator's manual for additionally required materials.

Assay

For use on the analyzer, treat the control serum in the system-compatible labeled bottles for analysis in the same way as the patient samples. Read the data encoded in the barcoded bottle labels and barcodes into the analyzer. Ensure the controls are at ambient temperature (20-25 °C) before measurement.

Run controls daily in parallel with patient samples, once per reagent kit, and whenever calibration is performed. The control intervals and limits should be adapted to each laboratory's individual requirements.

Additional controls may be tested in conformance with local, state, and/or federal regulations or accreditation requirements and your laboratory's quality control policy.

References

- Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
- Council Directive (2000/54/EC). Official Journal of the European Communities No. L262 from Oct. 17, 2000.

For further information, please refer to the appropriate operator's manual for the analyzer concerned, the respective application sheets, the product information, and the package inserts of all necessary components.

Limited License

The Elecsys Anti-HCV assay shall not be used by blood banks, donor centers, or other institutions which exclusively or predominantly use the test for the safety or screening of blood and blood products.

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PreciControl Anti-HCV (Draft)

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