Anti-HBc

Antibodies to hepatitis B core antigen (anti-HBc)

cobas®

REF 04927826 160 **100 tests**

• Indicates analyzers on which the kit can be used

Elecsys 2010
•

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-HBc immunoassay is for the *in vitro* qualitative determination of total antibodies to hepatitis B core antigen (anti-HBc) in human serum and plasma (lithium heparin, sodium citrate, K_2-EDTA) in adult patients with the symptoms of hepatitis or who may be at risk for hepatitis B (HBV) infection. The detection of total anti-HBc is indicative of a laboratory diagnosis for HBV infection. Further HBV serological marker testing is required to define the specific disease state. The Elecsys Anti-HBc immunoassay's performance has not been established for the monitoring of HBV disease or therapy.
The electrochemiluminescence immunoassay "ECLIA" is intended for use on the Elecsys 2010 immunoassay analyzer.

Summary

The hepatitis B virus consists of an outer envelope containing host-derived lipids and all S gene polypeptides, the large (L), middle (M), and small (S) surface proteins, also known as pre-S1, pre-S2 and HBsAg. The nucleocapsid contains core proteins HBcAg, a 3.2 kb, circular, partially double stranded viral DNA genome, an endogenous DNA polymerase (reverse transcriptase) enzyme, and protein kinase activity. The hepatitis core antigen comprises 183-185 amino acids.[1]
During an infection with the hepatitis B virus, antibodies to HBcAg are generally formed, which often persist for life. Anti-HBc appears shortly after the onset of infection with hepatitis B virus and can usually be detected in serum soon after the appearance of HBsAg. Anti-HBc antibodies persist both in persons who have recovered from a hepatitis B infection and in those who develop HBsAg-carrier status. Accordingly, they are an indicator of existing or past hepatitis B infection.[2]
In rare cases, an HBV infection can also run its course without the appearance of immunologically detectable anti-HBc (usually in immunosuppressed patients).[3]
Due to the long persistence of anti-HBc following a hepatitis B viral infection, screening for HBV infection may be accomplished by testing for the presence of hepatitis B core antibodies as long as those who test positive are further tested for both HBsAg and anti-HBs to differentiate infection from immunity.[4]
Determination of anti-HBc in association with other hepatitis B tests permits the diagnosis and monitoring of HBV infections. In the absence of other hepatitis B markers (HBsAg-negative persons), anti-HBc may be the only indication of an existing hepatitis B viral infection.[5]

Test principle

Competition principle. Total duration of assay: 27 minutes.
- 1st incubation: Pretreatment of 40 µL of sample with reducing agent (Patent No. US 6150113 for USA or EP 0 341 439 B1 for Europe).[6]
- 2nd incubation: After addition of HBcAg, a complex is formed with anti-HBc antibodies in the sample.
- 3rd incubation: After addition of biotinylated antibodies and ruthenium complex[a] -labeled antibodies specific for HBcAg, together with streptavidin-coated microparticles, the still-free binding sites on the HBc-antigens become occupied. The entire complex becomes bound to the solid phase via interaction of biotin and streptavidin.
- The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with ProCell. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.
- Results are determined automatically by the Elecsys 2010 software by comparing the electrochemiluminescence signal obtained from the reaction product of the sample with the signal of the cutoff value previously obtained by anti-HBc calibration.

a) Tris(2,2'-bipyridyl)ruthenium(II)-complex ($Ru(bpy)_3^{2+}$)

Reagents - working solutions

M	Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL: Streptavidin-coated microparticles 0.72 mg/mL; preservative.
R0	DTT (white cap), 1 bottle, 5 mL: 1,4-dithiothreitol 110 mmol/L; citrate buffer 50 mmol/L.
R1	HBcAg (gray cap), 1 bottle, 8 mL: HBcAg (*E. coli*, rDNA), > 25 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.
R2	Anti-HBcAg-Ab~biotin; anti-HBcAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL: Biotinylated monoclonal anti-HBc antibody (mouse) > 700 ng/mL; monoclonal anti-HBc antibody (mouse) labeled with ruthenium complex > 200 ng/mL; phosphate buffer 100 mmol/L, pH 7.4; preservative.
Cal1	Negative calibrator 1 (white cap), 2 bottles of 1.0 mL each: Human serum, preservative.
Cal2	Positive calibrator 2 (black cap), 2 bottles of 1.0 mL each: Anti-HBc (human) > 8 PEI U/mL[b] in human serum; preservative.

b) Paul-Ehrlich-Institute units

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.

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.[7]

All human material should be considered potentially infectious.

The calibrators have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (Cal1) and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The serum containing anti-HBc (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 just as carefully as a patient specimen. In the event of exposure the directives of the responsible health authorities should be followed.[7,8]

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 preparation.

Reagent handling

The reagents in the kit are ready for use and are supplied in bottles compatible with the system.

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Elecsys 2010 analyzer: The calibrators Cal1 and Cal2 should only be left on the analyzer during calibration at 20-25 °C. After use, close the bottles as soon as possible and store at 2-8 °C. Ensure that no calibration solution is trapped in the opened snap-cap. Because of possible evaporation effects, not more than 5 calibration procedures per calibrator bottle set should be performed. All information required for correct operation is read in via the respective reagent barcodes.

Storage and stability
Store at 2-8 °C.
Store the Elecsys Anti-HBc reagent kit (M, R0, R1, R2) **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
M, R0, R1, R2 after opening at 2-8 °C	8 weeks
on Elecsys 2010 after opening	4 weeks if stored continuously onboard or 8 weeks at 2-8 °C and up to 56 hours in total onboard if stored alternately in the refrigerator and on the analyzer.
calibrators:	
Cal1, Cal2 after opening at 2-8 °C	8 weeks
on Elecsys 2010 after opening	up to 5 hours

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

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

Serum collected using standard sampling tubes or tubes containing separating gel, lithium-heparin, K_2-EDTA and sodium-citrate 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.

Serum and plasma samples are stable for 5 days at 2-8 °C and serum samples are stable for 2 months at -20 °C. Serum and plasma samples may be frozen and thawed up to 4 times.

Attention! Particularly important for the Elecsys Anti-HBc assay: Frozen samples, samples containing precipitates, and samples for repeat measurements must be carefully centrifuged before performing the assay.

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.

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 analyzers should be measured within 2 hours.

Materials provided
See "Reagents - working solutions" section for reagents.
- 2 x 6 bottle labels

Materials required (but not provided)
- REF 04927931160, PreciControl Anti-HBc, for 8 x 1.3 mL each of PreciControl Anti-HBc 1 and 2
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010 analyzer

Accessories for Elecsys 2010 analyzers:
- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips
- 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.
Bring the cooled reagent 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 analyzers. Only keep open during calibration. All information necessary for calibration is encoded on the barcoded bottle labels and is read in automatically.
After calibration has been performed, discard.

Calibration
Traceability: This method has been standardized against the "HBc-Reference Material 82 (anti-HBc IgG)" of the Paul-Ehrlich-Institute, Langen (Germany).

Calibration frequency: Calibration must be performed once per reagent lot using Elecsys Anti-HBc Cal1 and Cal2, and fresh reagent (i.e. not more than 24 hours since the reagent kit was registered on the analyzer).

Renewed calibration is recommended as follows:
- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings with Elecsys PreciControl Anti-HBc reagents outside the specified limits
- more frequently when this is required by pertinent regulations

Range for the electrochemiluminescence signals (counts) for the calibrators:
Negative calibrator (Cal1): 42000-300000
Positive calibrator (Cal2): 100-3000

Quality control
For quality control, use Elecsys PreciControl Anti-HBc 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 the 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.

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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 > 1.1)
- "border"[c] (1.1 ≥ COI > 0.9)
- "reactive" (COI ≤ 0.9)

c) border = borderline

Interpretation of results:

Initial Elecsys Anti-HBc Assay Result			
COI	Result	Interpretation of results	Retest procedure
> 1.1	Non-reactive[d]	No antibodies to HBc were detected	No retest required
1.1 ≥ COI > 0.9	Border	Borderline zone (undetermined)	Retest in duplicate with the Elecsys Anti-HBc assay
≤ 0.9	Reactive	Antibodies to HBc detected	Follow CDC recommendations for supplemental testing

d) Please note: A negative anti-HBc result can indicate that the patient is either susceptible to HBV infection due to no past exposure, or is immune to HBV infection due to vaccination.

Final Elecsys Anti-HBc Assay Result			
Initial result (COI)	Result after retest (COI)	Final results	Interpretation of results
> 1.1	No retest required	NON-REACTIVE[e]	Antibodies to HBc were not detected; does not exclude the possibility of exposure to HBV
1.1 ≥ COI > 0.9	If 2 of the 3 results have a COI > 1.0	NON-REACTIVE	Antibodies to HBc were not detected; does not exclude the possibility of exposure to HBV
	If 2 of the 3 results have a COI ≤ 1.0	REACTIVE	Presumptive evidence of antibodies to HBc. Follow CDC recommendations for supplemental testing.
≤ 0.9	No retest required	REACTIVE	Presumptive evidence of antibodies to HBc. Follow CDC recommendations for supplemental testing.

e) Please note: A negative anti-HBc result can indicate that the patient is either susceptible to HBV infection due to no past exposure, or is immune to HBV infection due to vaccination.

Cutoff determination

The cutoff value was established with in-house studies by measuring a panel of 240 samples.
A Receiver Operator Curve (ROC) analysis was used to verify the cutoff.
Validation of the cutoff 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 HBc may not detect all infected individuals. A non-reactive test result does not exclude the possibility of exposure to HBV.

Do not use samples and controls stabilized with azide.

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.

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 Hepatitis C Virus (HCV), Hepatitis E Virus (HEV), Human T Cell Lymphotropic Virus (HTLV) and Human Immunodeficiency Virus (HIV).

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

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

The detection of anti-HBc antibodies indicates a present or past infection with hepatitis B 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 antigen used in this test.

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

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

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

Specific performance data

Representative performance data for the Elecsys Anti-HBc immunoassay run on the Elecsys 2010 analyzer are given below. Results obtained by individual laboratories may differ.

Precision

Within-laboratory precision

Repeatability and within-laboratory precision was determined on one Elecsys 2010 analyzer at one site 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 12 days (n = 48). Results are presented below.

Elecsys 2010 analyzer						
Sample	n	Mean	Repeatability[f]		Within-laboratory	
		COI[g]	SD	% CV	SD	CV
HS[h], negative	48	1.84	0.065	3.5	0.092	5.0
HS, high negative	48	1.03	0.016	1.5	0.045	4.3
HS, low positive	48	0.874	0.015	1.7	0.041	4.7
HS, positive	48	0.452	0.009	2.0	0.028	6.1
PC[i] A-HBC1	48	1.60	0.022	1.4	0.062	3.9
PC A-HBC2	48	0.378	0.010	2.8	0.042	11.2

f) Repeatability = within-run precision
g) COI = cutoff index
h) HS = human serum
i) PC = PreciControl

Reproducibility study

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 3 sites incorporating a 5 member panel consisting of four near cutoff human serum pools, one moderately reactive human serum pool, and PreciControl 1 and 2 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, between-run, between-day, between-lot, between-site and reproducibility.

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The overall imprecision data are summarized in the following table:

Elecsys Anti-HBc reproducibility on the Elecsys 2010 analyzer

Sample		HS1	HS2	HS3	HS4	HS5	PC[j]1	PC2
N		180	180	179	180	180	180	180
Mean	COI	1.178	1.094	0.916	0.825	0.611	1.666	0.379
Repeatability	SD	0.032	0.028	0.023	0.029	0.020	0.034	0.009
	% CV	2.7	2.6	2.5	3.5	3.3	2.0	2.5
Between-run	SD	0.027	0.023	0.019	0.015	0.013	0.038	0.011
	% CV	2.3	2.1	2.1	1.9	2.2	2.3	2.9
Between-day	SD	0.023	0.026	0.029	0.024	0.011	0.036	0.007
	% CV	1.9	2.4	3.1	2.9	1.7	2.1	2.0
Between-lot	SD	0.036	0.033	0.040	0.031	0.036	0.022	0.028
	% CV	3.1	3.0	4.4	3.8	5.8	1.3	7.4
Between-site	SD	0.000[k]	0.000[k]	0.000[k]	0.000[k]	0.000[k]	0.050	0.000[k]
	% CV	0.0	0.0	0.0	0.0	0.0	3.0	0.0
Reproducibility	SD	0.06	0.056	0.058	0.051	0.044	0.083	0.032
	% CV	5.1	5.1	6.3	6.2	7.3	5.0	8.5

j) PC = PreciControl A-HBc

k) 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, total protein and HAMA on the Elecsys Anti-HBc assay the following samples were tested, and the results are presented below:

Interferent tested	No interference up to:
Hemoglobin	0.8 g/dL
Bilirubin	25 mg/dL
Lipemia	1000 mg/dL
Biotin	30 ng/mL
Total protein	10 g/dL
HAMA	483 ng/mL

Drug interferences

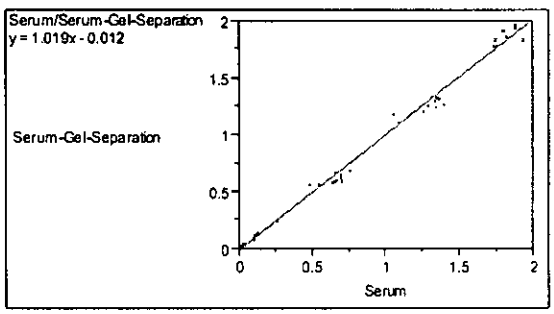
A drug interference study was performed with 18 common therapeutic drugs. Each drug was tested three-fold spiked into a negative and a low positive sample.

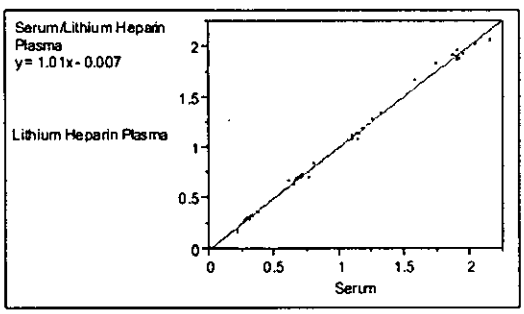
Each drug was found to be non-interfering at the following claimed concentrations:

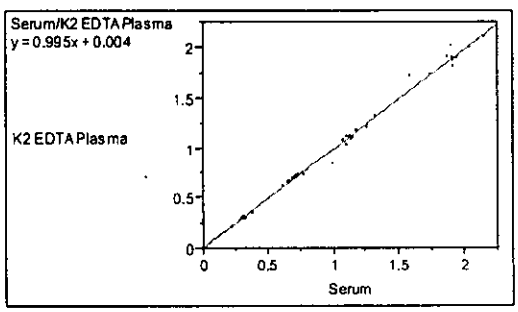
Compound	Concentration
Acetyl cysteine	150 mg/L
Ampicillin-Na	1000 mg/L
Ascorbic acid	300 mg/L
Ca-Dobesilate	200 mg/L
Cyclosporine	5 mg/L
Cefoxitin	2500 mg/L
Heparin	5000 U
Intralipid	10000 mg/L
Levodopa	20 mg/L
Methyldopa+ 1.5	20 mg/L
Metronidazole	200 mg/L
Phenylbutazone	400 mg/L
Tetracycline	50 mg/L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

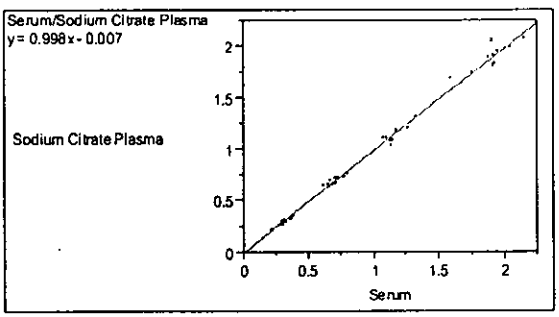
Matrix effects

Studies were conducted to evaluate the suitability of the following four types of blood collection tubes: serum/gel separation tubes, lithium heparin plasma, K_2-EDTA plasma and sodium citrate plasma. 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-HBc. The studies support the use of serum/gel separation tubes and the following plasma types: lithium heparin, K_2-EDTA and sodium citrate. The results are shown below:









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Analytical specificity

A study was conducted to evaluate the Elecsys Anti-HBc assay for potential cross-reactivity in specimens from individuals with medical conditions unrelated to hepatitis B infection. All specimens in the study were evaluated with the Elecsys Anti-HBc and the reference assay. The results are summarized in the following table:

Reactivity of the Elecsys Anti-HBc assay in individuals with medical conditions unrelated to hepatitis B infection

Category	anti-HBc Reference Assay				Total samples
	RX[l,m]		NR[n]		
	Elecsys Anti-HBc Assay				
	RX	NR	RX	NR	n
Anti-nuclear antibody (ANA)	0	0	0	15	15
Cytomegalovirus (anti-CMV positive)	2	0	0	10	12
Epstein-Barr Virus (anti-EBV positive	0	0	0	11	11
Hepatitis A Virus (anti-HAV positive)	0	0	0	9	9
HAV Vaccination	0	0	0	6	6
Hepatitis C Virus (anti-HCV positive)	3	0	1[o]	8	12
Hepatitis D Virus (anti-HDV positive)[p]	5	0	0	0	5
Hepatitis E Virus (anti-HEV positive)	6	0	2[o]	3	11
Human immunodeficiency virus (anti-HIV-1 positive)	5	0	1[o]	3	9
Herpes Simplex Virus (HSV) IgG	0	0	0	9	9
Human T-Cell Lymphotropic Virus (HTLV)	2	0	1[o]	9	12
Non-Viral Liver Disease	1	0	0	37	38
Parvovirus B_{19}infection	0	0	0	9	9
Rheumatoid Factor positive	0	0	0	11	11
Rubella	0	0	0	10	10
Syphilis (*T. pallidum*)	2	0	0	9	11
Toxoplasmosis IgG positive	2	0	0	6	8
Influenza vaccine recipients	0	0	0	10	10
HBV Vaccination	0	0	0	7	7
E. coli infection	6	0	0	6	12
Pregnancy	0	0	0	11	11
Varicella zoster (Anti-VZV)	1	0	0	7	8

l) RX = reactive

m) Samples that tested reactive for anti-HBc by the reference method were not further evaluated to establish the true hepatitis B infection status.

n) NR = non-reactive

o) A total of five discrepant results were observed with the Elecsys Anti-HBc assay: HCV (1/12); HEV (2/11); HTLV I/II (1/12); HIV (1/9)

p) The potential for cross-reactivity between anti-HDV reactive and, anti-HBc non-reactive samples has not been established.

Seroconversion sensitivity

Seroconversion sensitivity of the Elecsys Anti-HBc assay has been shown by testing 7 commercially-sourced seroconversion panels in comparison to a reference anti-HBc immunoassay. The comparison of the seroconversion detection between the two assays is summarized in the following table:

Elecsys Anti-HBc Days to evidence of HBV infection seroconversion panels

Panel ID	Days to change in reactivity of anti-HBc results				Difference in days to Elecsys Anti-HBc reactivity (Reference - Elecsys)
	Reference anti-HBc		Elecsys Anti-HBc		
	NR	RX	NR	RX	
11024	54	NC[q]	54	NC	N/A
6278	37	41	33	37	4
6281	36	41	36	41	0
9072	159	NC	159	NC	N/A
PHM933	16	144	16	144	0
PHM934	14	84	14	84	0
PHM935B	-[r]	128	-[r]	128	0

q) NC = no conversion

r) All bleeds were positive for anti-HBc.

The Elecsys Anti-HBc assay was reactive in the same bleed as the reference assay in four of the seven panels tested. The Elecsys Anti-HBc assay was reactive earlier than the reference assay in one panel, while seroconversion never occurred in either assay in two panels.

Summary of clinical performance

A multicenter study was conducted to evaluate the ability of the Elecsys Anti-HBc assay to detect anti-HBc antibodies in specimens from an intended use population.

Of the 1526 specimens tested in the Elecsys Anti-HBc clinical study, 959 specimens were obtained from individuals at risk of HBV infection due to lifestyle, behavior, occupation, disease state or known exposure event; and 567 specimens were obtained from individuals with signs and symptoms of a hepatitis infection.

The 1526 specimens were collected from ten collection sites located in Florida (44.4 %), California (47.8 %), Georgia (5.90 %), and New Jersey (1.90 %). A demographic summary of the overall specimen population by age and race/ethnic group is provided in the following tables.

Demographic summary of overall specimen population by age

	Overall		Asymptomatic		Symptomatic	
Age Group	n	%	n	%	n	%
21 to 30	273	17.9	164	17.1	109	19.2
31 to 40	308	20.2	212	22.1	96	16.9
41 to 50	558	36.6	359	37.4	199	35.1
51 to 60	328	21.5	196	20.4	132	23.3
61 to 70	51	3.34	23	2.40	28	4.94
71 to 80	8	0.52	5	0.52	3	0.53
Total	1526	100	959	100	567	100

Demographic summary of overall specimen population by race

Race	Group (n)	Percent (%)
African American/Black	731	47.9
American Indian/Alaska Native	10	0.66
Asian	5	0.33
Caucasian/White	699	45.8
Pacific Islander	4	0.26
Other	18	1.18
Unknown	59	3.87
Total	1526	100

Of the 1526 at risk subjects, 431 (28.2 %) were female and 1095 (71.8 %) were male. The mean age of the subjects was 42.6 years (age range: 21 to 79 years). Testing of the specimens was performed at 3 clinical testing sites located in St. Louis, MO, Baltimore, MD, and Boston, MA.

Anti-HBc

Antibodies to hepatitis B core antigen (anti-HBc)

cobas®

Results by specimen classification

HBV classifications were determined based on the constellation of test results from an FDA-approved HBV marker panel. Using the reference anti-HBc assay, the specimens were assigned an HBV status based on the algorithm provided in the following table:

Serological Classification by FDA-Approved HBV Panel

	HBsAg	HBeAg	AntiHBc IgM	Reference anti-HBc	Anti-HBe	Anti-HBs
Acute	(+)	(+)	(-)	(-)	(-)	(-)
Acute	(+)	(+) or (-)	eq	(+)	(+)	(-)
Acute	(+)	(+)	(+)	(+)	(-), (+), nd/qns	(-)
Acute	(+)	(-)	(+)	(+)	(+)	(-)
Acute	(+)	qns	(+)	(+)	(+)	(-)
Acute	(+)	(+)	(+)	(+)	(-)	eq
Acute (Late)	(+)	(-)	(+)	(+)	(+)	(+)
Chronic	(+) >6 mo.					
Chronic	(+) >6 mo.	(-)	(-)	(+)	(+), eq, (+)	(-)
Chronic	(+) >6 mo.	(+)	eq	(+)	(-)	(-)
Chronic	(+)	(+)	(-)	(+)	(-)	(-)
Chronic	(+)	(-)	(-)	(+)	(+), eq, (+)	(-)
Chronic	(+)	(+)	(+)	(+)	(-)	(+)
Early Recovery	(-)	(-)	eq	(+)	(+)	(+) or eq
Early Recovery	(-)	(-)	(-)	(+)	(+)	(+)
Early Recovery	(-)	(-)	(+)	(+)	(+), eq, (-), qns	(-)
Recovery	(-)	(-)	(-)	(+)	(+)	(+) or eq
Recovery	(-)	(-)	(-)	(+)	eq	(+)
Recovery	(-)	(-)	(-)	(-)	(+)	(+)
Recovered or Immune Due to Natural Infection	(-)	(-)	(-)	(+)	(-)	(+) or eq
HBV Vaccine Response	(-)	(-)	(-)	(-)	(-)	(+)
HBV Vaccine Response (?)	(-)	(-)	(-)	(-)	(-)	eq
Not Previously Infected	(-)	(-)	(-)	(-)	(-)	(-)
Not Previously Infected	rr uncnf	(-)	(-)	(-)	(-)	(-)
Not Interpretable	(+)	(+)	nd	(+)	(+) or (-)	(-)
Not Interpretable	(+)	(-)	eq	(+)	(-)	(+)
Not Interpretable	(-)	(-)	(-)	(-)	(+)	(-)
Not Interpretable	(-)	(-)	(-)	(+)	qns	(+)
Not Interpretable	(-)	(+)	(-)	(-)	(-)	(+) or (-)
Not Interpretable	qns	(-)	(-)	(-)	(-)	(-)

nd = not detected

eq = equivocal or indeterminate or borderline

rr uncnf = repeatedly reactive: did not confirm

qns = incomplete or unconfirmed

Results of HBV classification

Asymptomatic at risk population

The following table compares Elecsys Anti-HBc results on the Elecsys 2010 analyzer with the results obtained with the reference anti-HBc assay for specimens which have been serologically classified in the asymptomatic at risk of HBV infection cohort.

Comparison of Elecsys Anti-HBc Assay on the Elecsys 2010 Analyzer to the Reference Assay Results by HBV Classification in the Asymptomatic At Risk Cohort

HBV Classification	Reference anti-HBc Assay Results				Total
	+		-		
	Elecsys Anti-HBc Test Result				
	+	-	+	-	
Acute	7	0	0	1	8
Chronic	27	1	0	0	28
Early Recovery	61	1	0	0	62
Recovery	138	0	1	0	139
Recovered	91	6	0	0	97
HBV Vaccination	0	0	6	183	189
Not Previously Infected	0	0	6	426	432
Not Interpretable	1	0	0	3	4
Total	325	8	13	613	959

The table below summarizes the percent agreement between the Elecsys Anti-HBc assay and the reference assay with clinically classified samples in the asymptomatic at risk cohort. The table also provides the upper and lower 95 % exact confidence bounds.

Positive and Negative Percent Agreement in the Asymptomatic At Risk Population

HBV Classification	Positive % Agreement (n/N)	95 % Exact Confidence Interval	Negative % Agreement (n/N)	95 % Exact Confidence Interval
Acute	100 (7/7)	59.0-100	100 (1/1)	2.50-100
Chronic	96.4 (27/28)	81.7-99.9	0.00 (0/0)	0.00-100
Early Recovery	98.4 (61/62)	91.3-100	0.00 (0/0)	0.00-100
Recovery	100 (138/138)	97.4-100	0.00 (0/1)	0.00-97.5
Recovered	93.8 (91/97)	87.0-97.7	0.00 (0/0)	0.00-100
HBV Vaccination	0.00 (0/0)	0.00-100	96.8 (183/189)	93.2-98.8
Not Previously Infected	0.00 (0/0)	0.00-100	98.6 (426/432)	97.0-99.5
Not Interpretable	100 (1/1)	2.50-100	100 (3/3)	29.2-100
Total	97.6 (325/333)	95.3-99.0	97.9 (613/626)	96.5-98.9

The positive percent agreement between the Elecsys Anti-HBc assay results and the HBV infected status for the asymptomatic at risk for HBV infection population (n = 959) was 97.6 % (325/333) with a 95 % confidence interval of 95.3 - 99.0 %. The negative percent agreement between the Elecsys Anti-HBc assay results with the not HBV infected status was 97.9 % (613/626) with a 95 % confidence interval of 96.5 - 98.9 %.

Symptomatic at risk population

The following table compares Elecsys Anti-HBc results on the Elecsys 2010 analyzer with the results obtained with the reference anti-HBc assay for specimens which have been serologically classified in the symptomatic at risk for HBV infection cohort.

Anti-HBc

Antibodies to hepatitis B core antigen (anti-HBc)

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Comparison of Elecsys Anti-HBc Assay on the Elecsys 2010 Analyzer to the Reference Assay Results by HBV Classification in the Symptomatic At Risk Cohort

HBV Classification	Reference anti-HBc Assay Results				Total
	+		-		
	Elecsys Anti-HBc Test Result				
	+	-	+	-	
Acute	48	0	0	0	48
Chronic	9	1	0	0	10
Early Recovery	38	2	0	0	40
Recovery	70	0	0	0	70
Recovered	33	1	0	0	34
HBV Vaccination	0	0	6	129	135
Not Previously Infected	0	0	4	221	225
Not Interpretable	3	0	1	1	5
Total	201	4	11	351	567

The table below summarizes the percent agreement between the Elecsys Anti-HBc assay on the Elecsys 2010 analyzer and the reference assay with clinically classified samples in the symptomatic at risk cohort. The table also provides the upper and lower 95 % exact confidence bounds.

Positive and Negative Percent Agreement in the Symptomatic At Risk Population

HBV Classification	Positive % Agreement (n/N)	95 % Exact Confidence Interval	Negative % Agreement (n/N)	95 % Exact Confidence Interval
Acute	100 (48/48)	93.6-100	0.00 (0/0)	0.00-100
Chronic	90.0 (9/10)	55.5-99.8	0.00 (0/0)	0.00-100
Early Recovery	95.0 (38/40)	83.1-99.4	0.00 (0/0)	0.00-100
Recovery	100 (70/70)	94.9-100	0.00 (0/0)	0.00-100
Recovered	97.1 (33/34)	84.7-99.9	0.00 (0/0)	0.00-100
HBV Vaccination	0.00 (0/0)	0.00-100	95.6 (129/135)	90.6-98.4
Not Previously Infected	0.00 (0/0)	0.00-100	98.2 (221/225)	95.5-99.5
Not Interpretable	100 (3/3)	29.2-100	50.0 (1/2)	1.26-98.7
Total	98.1 (201/205)	95.1-99.5	97.0 (351/362)	94.6-98.5

The positive percent agreement between the Elecsys Anti-HBc assay results and the HBV infected status for the symptomatic at risk population (n = 567) was 98.1 % (201/205) with a 95 % confidence interval of 95.1 - 99.5 %. The negative percent agreement between the Elecsys Anti-HBc assay results with the not HBV infected status was 97.0 % (351/362) with a 95 % confidence interval of 94.6 - 98.5 %.

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1. Sällberg M, et al. Characterisation of a Linear Binding Site for a Monoclonal Antibody to Hepatitis B Core Antigen. J Med Virol 1991;33:248-252.
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5. Gerlich WH, Caspari G, Uy A, Thomssen R. A critical appraisal of anti-HBc, HBV DNA and anti-HCV in the diagnosis of viral hepatitis. Biotest Bulletin 1991;4:283-293.
6. Patent owned by Abbott Laboratories, USA.
7. Occupational Safety and Health Standards: bloodborne pathogens. (29 CFR Part 1910.1030). Fed. Register.
8. 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.

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PreciControl Anti-HBc

Elecsys 2010 analyzer

REF 04927931 160

16 x 1.3 mL

Intended use

Elecsys PreciControl Anti-HBc is used for quality control of the Elecsys Anti-HBc immunoassay on the Elecsys 2010 analyzer.

Summary

Elecsys PreciControl Anti-HBc 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 Elecsys Anti-HBc immunoassays.

Reagents - working solutions

- **PC A-HBC1: 8 bottles, each containing 1.3 mL of control serum Human serum, negative for anti-HBc; buffered and preserved with 0.4 % Bronidox L.**
  Target range for the cutoff index: 1.05-3.0 COI.
  The exact ranges, (target value ± 22 %), 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.
- **PC A-HBC2: 8 bottles, each containing 1.3 mL of control serum Anti-HBc antibodies (human) approx. 1 U/mL (Paul-Ehrlich-Institute units) in human serum; buffered with HEPES and preserved with 0.4 %.Bronidox L.**
  Target range for the cutoff index: 0.14-0.87 COI.

The exact ranges, (target value ± 44 %), 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-HBc manufactured, the PreciControls are run in duplicate in at least 8 series on MODULAR ANALYTICS and Elecsys 2010 analyzers. The target value of each PreciControl is defined as the mean value obtained over at least 8 determinations (duplicate runs on at least 2 analyzers) of the respective PreciControl.
Traceability of the Elecsys Anti-HBc assay is given in the package insert of the respective Elecsys Anti-HBc assay. Control values have not been established for assays other than the Elecsys Anti-HBc 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 (PC A-HBC1 only) and antibodies to HCV and HIV.
The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.
The serum containing anti-HBc used for the positive control (PC A-HBC2) was inactivated using Î²-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 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.

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-HBc, 2 barcode cards, control barcode sheet

Materials required (but not provided)

- Elecsys 2010 analyzer and assay reagents. See test 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

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

2. 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-HBc 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.
