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Anti-HBs

cobas®

Antibody to hepatitis B surface antigen (anti-HBs)

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
11820524 122	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of human antibodies to the hepatitis B surface antigen (HBsAg) in human serum and plasma.

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

Summary

Anti-HBs is a specific (generally IgG) antibody that is directed against the hepatitis B surface antigen.[1] Anti-HBs can be formed following a hepatitis B infection or after hepatitis B vaccination. Antibodies are formed against the HBsAg determinant a, which is common to all subtypes, and against subtype-specific determinants.[2]

Anti-HBs tests are used within the scope of hepatitis B vaccination to check the necessity and success of vaccination.[3,4,5] In addition, anti-HBs tests are used to monitor the course of disease following acute hepatitis B infection.[3]

The Elecsys Anti-HBs assay uses a mixture of purified antigens of the HBsAg subtypes ad and ay from human serum.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: Anti-HBs in the sample (40 µL), biotinylated HBsAg (ad/ay), and HBsAg (ad/ay) 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 via a calibration curve which is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode.

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

Reagents - working solutions

The reagent rackpack (M, R1, R2) is labeled as A-HBS.

M Streptavidin-coated microparticles (transparent cap), 1 bottle, 6.5 mL:
Streptavidin-coated microparticles 0.72 mg/mL; preservative.

R1 HBsAg~biotin (gray cap), 1 bottle, 10 mL:
Biotinylated HBsAg (ad/ay) human, > 0.5 mg/L; MES[b)] buffer 85 mmol/L, pH 6.5; preservative.

R2 HBsAg~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 8 mL:
HBsAg (ad/ay) human, labeled with ruthenium complex > 0.3 mg/L; MES buffer 85 mmol/L, pH 6.5; preservative.

b) MES = 2-morpholino-ethane sulfonic acid

A-HBS Cal1 Calibrator 1 (white cap), 2 bottles of 1.3 mL each:
Anti-HBs (human) in human serum; preservative.

A-HBS Cal2 Calibrator 2 (black cap), 2 bottles of 1.3 mL each:
Anti-HBs (human) in human serum; preservative.

Precautions and warnings

For in vitro diagnostic use.
Exercise the normal precautions required for handling all laboratory reagents.
Disposal of all waste material should be in accordance with local guidelines.
Safety data sheet available for professional user on request.

This kit contains components classified as follows in accordance with the Regulation (EC) No. 1272/2008:

n-Octyl-N,N-dimethyl-3-ammonio-1-propanesulfonate

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

All human material should be considered potentially infectious. The calibrators (A-HBS Cal1 and A-HBS Cal2) have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg and antibodies to HCV and HIV. The testing methods applied were FDA-approved or cleared in compliance with the European Directive 98/79/EC, Annex II, List A.

The HBsAg starting material used was inactivated prior to labeling with biotin or ruthenium by heating to 60 °C for 15 hours. In addition, any virus particles remaining were removed by ultracentrifugation.

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.[6,7]

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

Reagent handling

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

Elecsys 2010 and **cobas e** 411 analyzers: The calibrators should only be left on the analyzers during calibration at 20-25 °C. After use, close the bottles as soon as possible and store upright at 2-8 °C.

Due to possible evaporation effects, not more than 5 calibration procedures per bottle set should be performed.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: Unless the entire volume is necessary for calibration on the analyzers, transfer aliquots of the 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.

Please note: Both the vial labels, and the additional labels (if available) contain 2 different barcodes. The barcode between the yellow markers is for **cobas** 8000 systems only. If using a **cobas** 8000 system, please turn the vial cap 180° into the correct position so the barcode can be read by the system. Place the vial on the instrument as usual.

Storage and stability

Store at 2-8 °C.

Do not freeze.

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

Stability of the reagent rackpack	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks

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Stability of the reagent rackpack	
on MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602	8 weeks
on Elecsys 2010 and **cobas e** 411	4 weeks

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

Store calibrators **upright** in order to prevent the calibrator solution from adhering to the snap-cap.

Specimen collection and preparation

Only the specimens listed below were tested and found acceptable.

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

K_3-EDTA plasma.

Criterion: Recovery within 90-110 % of serum value or slope 0.9-1.1 + intercept within < ± 2x analytical sensitivity (LDL) + coefficient of correlation > 0.95.

If plasma treated with lithium heparin, sodium citrate or sodium fluoride/potassium oxalate is used, the values obtained are 25 % lower than those obtained from serum.

Do not use lithium heparin plasma tubes containing separating gel.

Stable for 6 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen 6 times.

The sample types listed were tested with a selection of sample collection tubes that were commercially available at the time of testing, i.e. not all available tubes of all manufacturers were tested. Sample collection systems from various manufacturers may contain differing materials which could affect the test results in some cases. When processing samples in primary tubes (sample collection systems), follow the instructions of the tube manufacturer.

Centrifuge samples containing precipitates and frozen samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Ensure the samples, calibrators and controls are at 20-25 °C prior to measurement.

Due to possible evaporation effects, samples and calibrators on the analyzers should be analyzed/measured within 2 hours.

Materials provided

See "Reagents – working solutions" section for reagents.

- 2 x 6 bottle labels

Materials required (but not provided)

- REF 11876317122, PreciControl Anti-HBs, for 8 x 1.3 mL each of PreciControl Anti-HBs 1 and 2
- REF 11732277122, Diluent Universal, 2 x 16 mL sample diluent or REF 03183971122, Diluent Universal, 2 x 36 mL sample diluent
- REF 11776576322, CalSet Vials, 2 x 56 empty snap-cap bottles
- General laboratory equipment
- Elecsys 2010, MODULAR ANALYTICS E170 or **cobas e** analyzer

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

- REF 11662988122, ProCell, 6 x 380 mL system buffer
- REF 11662970122, CleanCell, 6 x 380 mL measuring cell cleaning solution
- REF 11930346122, Elecsys SysWash, 1 x 500 mL washwater additive
- REF 11933159001, Adapter for SysClean
- REF 11706802001, Elecsys 2010 AssayCup, 60 x 60 reaction vessels
- REF 11706799001, Elecsys 2010 AssayTip, 30 x 120 pipette tips

Accessories for MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers:

- REF 04880340190, ProCell M, 2 x 2 L system buffer
- REF 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- REF 03023141001, PC/CC-Cups, 12 cups to prewarm ProCell M and CleanCell M before use
- REF 03005712190, ProbeWash M, 12 x 70 mL cleaning solution for run finalization and rinsing during reagent change
- REF 12102137001, AssayTip/AssayCup Combimagazine M, 48 magazines x 84 reaction vessels or pipette tips, waste bags
- REF 03023150001, WasteLiner, waste bags
- REF 03027651001, SysClean Adapter M

Accessories for all analyzers:

- REF 11298500316, 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.

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.

After calibration has been performed, store the calibrators at 2-8 °C or discard (MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

Calibration

Traceability: This method has been standardized against the 1st WHO Reference Standard 1977.

Every Elecsys Anti-HBs 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 A-HBS Cal1 and A-HBS Cal2.

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

Renewed calibration is recommended as follows:

- after 1 month (28 days) when using the same reagent lot
- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings with PreciControl Anti-HBs outside the defined limits
- more frequently when this is required by pertinent regulations

Range (in IU/L) for the calibrators: 4-15 for calibrator 1 (A-HBS Cal1) and 350-600 for calibrator 2 (A-HBS Cal2).

Quality control

For quality control, use PreciControl Anti-HBs.

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

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limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

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

Calculation

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

Limitations - interference

The assay is unaffected by icterus (bilirubin < 513 µmol/L or < 30 mg/dL), hemolysis (Hb < 0.93 mmol/L or < 1.5 g/dL), lipemia (Intralipid < 1500 mg/dL) and biotin (< 123 nmol/L or < 30 ng/mL).

Criterion: Recovery within ± 10 % of initial value.

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

No interference was observed from rheumatoid factors up to a concentration of 2100 IU/mL.

A high-dose hook effect[c)] may be observed for samples with anti-HBs concentrations > 150000 IU/L. In rare cases a high-dose hook effect < 150000 IU/L cannot be excluded. In case of an unexpected low result, i.e. after re-vaccination, the sample should be diluted 1:100 (refer to chapter "Dilution") and tested again.

In vitro tests were performed on 17 commonly used pharmaceuticals. No interference with the assay was found.

In rare cases, interference due to extremely high titers of antibodies to streptavidin and ruthenium can occur. The test contains additives which minimize these effects.

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers: In case the Elecsys HBsAg II/Anti-HBs and HBeAg/Anti-HBe assay combinations are processed, make sure that these assays are entered in the "Special Wash" section of the system software and "Step1" (wash execute) is checked. Please refer to the operator's manual.

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

c) High-dose hook effect: A sample with a true concentration clearly above the measuring range, but found within the measuring range.

Limits and ranges

Measuring range

2.00-1000 IU/L (defined by the detection limit and the maximum of the master curve). Values below the detection limit are reported as < 2.00 IU/L. Values above the measuring range are reported as > 1000 IU/L (or up to 100000 IU/L for 100-fold diluted samples).

Detection limit

≤ 2.0 IU/L

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

Sensitivity in diluted samples

Two human sera reactive for anti-HBs were stepwise diluted with serum negative for anti-HBs and assayed in duplicate with the Elecsys Anti-HBs assay and a commercial comparison test:

HS 1 Dilution	Elecsys Anti-HBs IU/L	Comparison test IU/L	HS 2 Dilution	Elecsys Anti-HBs IU/L	Comparison test IU/L
1	> 1000	> 150	1	> 1000	> 150
1:100	> 1000	> 150	1:100	> 1000	> 150
1:500	310	> 150	1:500	261	> 150
1:1000	161	109	1:1000	136	99.0
1:2500	69.5	34.3	1:2500	59.0	36.5
1:5000	36.5	17.9	1:5000	31.8	17.1
1:10000	19.8	8.4	1:10000	16.5	9.0
1:20000	10.3	4.3	1:20000	8.5	4.3

Dilution

Samples with anti-HBs concentrations above the measuring range can be diluted with Diluent Universal. The recommended dilution is 1:100 (either automatically by the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** analyzers or manually). The concentration of the diluted sample must be > 10 IU/L.

After manual dilution, multiply the result by the dilution factor.

After dilution by the analyzers, the MODULAR ANALYTICS E170, Elecsys 2010 and **cobas e** software automatically takes the dilution into account when calculating the sample concentration.

Manual dilution can also be made with negative human serum.

Note: Antibodies to HBsAg are heterogenous. In some isolated cases, this may lead to non-linear dilution behavior.

Expected values

Interpretation of the results

Samples with concentrations < 10 IU/L are considered non-reactive in the Elecsys Anti-HBs assay.

Samples with concentrations ≥ 10 IU/L are considered reactive in the Elecsys Anti-HBs assay.

Note: Due to the diversity of the antibodies, the measured anti-HBs value can vary depending on the testing procedure used. Results obtained from a single sample using tests from different manufacturers can therefore differ by up to a factor of 4 (or even a factor of 10 in rare cases).[8] If there is a change in the assay procedure used during the monitoring of vaccination protection, then the anti-HBs values obtained upon changing over to the new procedure must be confirmed by parallel measurements with both methods.

Vaccination strategies in certain risk groups are based on the measured anti-HBs concentration.[4] Respective recommendations are given by national or regional guidelines.

Specific performance data

Representative performance data on the analyzers are given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, human sera and controls.

Elecsys 2010 and **cobas e** 411 analyzers						
	Repeatability[d)]			**Intermediate precision**[e)]		
Sample	Mean IU/L	SD IU/L	CV %	Mean IU/L	SD IU/L	CV %
HS[f)], negative	8.7	0.57	6.6	7.73	0.76	9.9
HS, weakly positive	15.4	0.52	3.4	12.9	1.42	11.0
HS, positive	603	9.30	1.5	605	20.6	3.4
A-HBS Cal1	10.7	0.67	6.3	11.0	1.79	16.2
A-HBS Cal2	498	14.1	2.8	514	18.4	3.6

d) Repeatability = within-run precision (n = 21)

e) Intermediate precision = between-run (n = 10)

f) HS = human serum, negative

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability[g)]			**Intermediate precision**[h)]		
Sample	Mean IU/L	SD IU/L	CV %	Mean IU/L	SD IU/L	CV %
HS, negative	< 2.00	-	-	< 2.00	-	-
HS, borderline	9.42	0.41	4.3	8.52	1.18	13.9

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MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers						
	Repeatability[g]			**Intermediate precision**[h]		
Sample	Mean IU/L	SD IU/L	CV %	Mean IU/L	SD IU/L	CV %
HS, positive	916	11.2	1.2	910	37.3	4.1
PC A-HBS1[i]	< 2.00	-	-	< 2.00	-	-
PC A-HBS2	89.6	0.99	1.1	86	4.87	5.7

g) Repeatability = within-run precision (n = 21)

h) Intermediate precision = within-laboratory (modified protocol (EP5-A) of the CLSI (Clinical and Laboratory Standards Institute): 6 times daily for 10 days (n = 60))

i) PC = PreciControl

Analytical specificity

No cross-reactions with HAV, HCV, HEV, CMV, EBV, HIV, Rubella, Toxoplasma gondii, and Treponema pallidum were observed.

Measurements were performed on each of the pathogens listed above using ≥ 5 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens.

Clinical sensitivity

Samples from various patient groups found to be reactive in a comparison test were measured with the Elecsys Anti-HBs assay and confirmed as anti-HBs-positive by additional HBV-tests.

Characterization of samples Sample groups	Number tested	Elecsys Anti-HBs reactive	Anti-HBs comparison tests reactive	Sensitivity %
Convalescents positive for: a-HBc, a-HBe, a-HBs	203	201	203	99.0
Drug addicts positive for: a-HBc, a-HBs	53	52	52	100
Hospitalized patients positive for: a-HBc, a-HBs	86	78	80	97.5
Vaccinated persons	133	131	131	100
Total	475	462	466	99.0

Clinical specificity

Samples from blood donors which had not been selected and hospitalized patients were used to determine the specificity.

Group	Number tested	Elecsys Anti-HBs false positive	Specificity %
Blood donors a-HBs < 10 IU/L (comparison test)	1469	3	99.8
Hospitalized patients negative for: a-HBs, a-HBc (comparison tests)	297	0	100

References

1 Lander JJ, Holland PV, Alter HJ, et al. Antibody to hepatitis-associated antigen. Frequency and patterns of response as detected by radioimmunoprecipitation. J Am Med Assoc 1972;220:1079-1082.

2 Gold JW, Alter HJ, Holland PV, et al. Passive hemagglutination assay for antibody to subtypes of hepatitis B antigen. J Immunol 1974;112;1100-1106.

3 Jilg W, Schmidt M, Deinhardt F. Immune Response to Hepatitis B Revaccination. J Med Virol 1988;24:377-384.

4 European Consensus Group on Hepatitis B immunity: Are booster immunisations needed for lifelong hepatitis B immunity? Lancet 2000;355:561-565.

5 Hoofnagle JH, Di Bisceglie AM. Serologic Diagnosis of Acute and Chronic Viral Hepatitis, Seminars in Liver Disease 1991;11/2:73-83.

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

7 Directive 2000/54/EC of the European Parliament and Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work.

8 Bornhak H, Jilg W, Hüdig H, et al. Quantitation of anti-HBs in solid phase immunoassays. What influences the results? Aus: Virushepatitis A bis E. Diagnose, Therapie Prophylaxe. Kilian Verlag, ISBN: 3-9803688-1-5, 1994;212-221.

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

A point (period/stop) is always used in this Method Sheet as the decimal separator to mark the border between the integral and the fractional parts of a decimal numeral. Separators for thousands are not used.

Symbols

Roche Diagnostics uses the following symbols and signs in addition to those listed in the ISO 15223-1 standard.

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

CE 0123

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