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HBsAg II quant

cobas®

Hepatitis B surface antigen quantitative determination

REF	Σ	SYSTEM
		Elecsys 2010
		MODULAR ANALYTICS E170
05957435 190	100	**cobas e** 411
		cobas e 601
		cobas e 602

English

Intended use

Immunoassay for the in vitro quantitative determination of hepatitis B surface antigen (HBsAg) in confirmed HBsAg positive 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

The hepatitis B surface antigen (HBsAg), a polypeptide of varying size, is a component of the external envelope of the hepatitis B virus particle (HBV).[1] In addition to the intact infectious viral particles, the blood of persons infected with HBV contains large amounts of non-infectious particles which consist only of an outer coat containing HBsAg.[2] After infection, HBsAg is the first immunological marker detectable in serum and is usually present weeks to months before the onset of clinical symptoms and the appearance of other biochemical markers. In the case of acute HBV infection with recovery, HBsAg is no longer detectable in serum 6 months after its appearance at the latest.[3,4] If HBsAg persists for more than 6 months after acute hepatitis, the presence of chronic hepatitis B (CHB) infection must be assumed. A CHB patient with elevated aminotransferase levels, high HBV DNA viral load, and histological abnormalities will be considered for therapy.[5] Two different treatment strategies are applicable: treatment of finite duration with pegylated interferon alpha and long-term treatment with nucleoside/nucleotide analogs (NUCs). The current standard for monitoring of these therapies is HBV DNA quantification. Therapy must reduce HBV DNA to as low a level as possible, ideally below the lower limit of detection of real-time PCR assays (10-15 IU/mL), to ensure a degree of virological suppression that will then lead to biochemical remission, histological improvement and prevention of complications. However, the ideal end-point of therapy is sustained HBsAg loss with or without seroconversion to anti-HBs. This is associated with a complete immune control of the virus and remission of the activity of chronic hepatitis B and an improved long-term outcome. Therefore, several recent studies suggest to monitor pegylated interferon alpha therapy with HBsAg quantification in addition to HBV DNA quantification.[6,7,8,9,10,11] Furthermore, HBsAg quantitation might have the potential to predict sustained virological response and HBsAg loss. Further studies are necessary to confirm these findings and to determine the importance of HBsAg quantification for monitoring of CHB patients under NUC therapy[12] as well as monitoring of CHB patients without therapy to identify inactive carrier in a one time measurement using HBsAg quantification and HBV DNA quantification.[13,14,15]

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

- 1st incubation: 50 µL of sample, two biotinylated monoclonal anti-HBsAg antibodies, and a mixture of monoclonal anti-HBsAg antibody and polyclonal anti-HBsAg antibodies labeled with a ruthenium complex[a)] 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 HBSAG-QN.

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

R1 Anti-HBsAg-Ab~biotin (gray cap), 1 bottle, 8 mL:
Two biotinylated monoclonal anti-HBsAg antibodies (mouse) > 0.5 mg/L; phosphate buffer 100 mmol/L, pH 7.5; preservative.

R2 Anti-HBsAg-Ab~$Ru(bpy)_3^{2+}$ (black cap), 1 bottle, 7 mL:
Monoclonal anti-HBsAg antibody (mouse), polyclonal anti-HBsAg antibodies (sheep) labeled with ruthenium complex > 1.5 mg/L; phosphate buffer 100 mmol/L, pH 8.0; preservative.

HBSAG-QN Cal1	Negative calibrator 1 (white cap), 2 bottles of 1.3 mL each: Human serum; preservative.
HBSAG-QN Cal2	Positive calibrator 2 (black cap), 2 bottles of 1.3 mL each: HBsAg approx. 0.5 IU/mL in human serum; preservative.
HBSAG-QN DilHepB	2 bottles of 36 mL each (white cap): Human serum negative for HBsAg and anti-HBs, buffered, pH 6.5; 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.

All human material should be considered potentially infectious.

The calibrators and HBSAG-QN Dil HepB have been prepared exclusively from the blood of donors tested individually and shown to be free from HBsAg (HBSAG-QN Cal1 and HBSAG-QN Dil HepB 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 HBsAg (HBSAG-QN Cal2) was inactivated using β-propiolactone and UV-radiation.

However, as no inactivation or testing method can rule out the potential risk of infection with absolute certainty, the material should be handled with the same level of care as a patient specimen. In the event of exposure, the directives of the responsible health authorities should be followed.[16,17]

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

Pre-dilution of samples is mandatory according to the test algorithm (see "Dilution" section).

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 and HBSAG-QN Dil HepB	
unopened at 2-8 °C	up to the stated expiration date
after opening at 2-8 °C	8 weeks
on the analyzers	8 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 in a sufficient number and found acceptable.

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

Li-heparin, Na-heparin, EDTA- and citrate-plasma.

Criterion: slope 1.00 ± 0.1 + intercept ≤ ± 0.5 IU/mL + coefficient of correlation ≥ 0.95.

Stable for 7 days at 2-8 °C, 3 months at -20 °C. The samples may be frozen 5 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 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 04687876190, PreciControl HBsAg II, for 8 x 1.3 mL each of PreciControl HBsAg II 1 and 2
- 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. **Pre-dilution of samples is mandatory according to the test algorithm (see "Dilution" section).** 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 NIBSC standard (code number: 00/588; WHO Second International Standard for HBsAg, subtype adw2, genotype A; IU/mL).

Every Elecsys HBsAg II quant 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 HBSAG-QN Cal1 and HBSAG-QN Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using HBSAG-QN Cal1, HBSAG-QN 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

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- after 7 days (when using the same reagent kit on the analyzer)
- as required: e.g. quality control findings outside the defined limits

Quality control

For quality control, use PreciControl HBsAg II.

Controls for the various concentration ranges should be run individually at least once every 24 hours when the test is in use, once per reagent kit, and following each calibration.

The control intervals and limits should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined limits. Each laboratory should establish corrective measures to be taken if values fall outside the defined limits.

If necessary, repeat the measurement of the samples concerned.

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

Calculation

The analyzer automatically calculates the analyte concentration (IU/mL) based on the measurement of HBSAG-QN Cal1 and HBSAG-QN Cal2. In case of a manual pre-dilution, the dilution factor needs to be accounted for manual calculation of the final result.

Limitations - interference

The assay is unaffected by icterus (bilirubin < 684 µmol/L or < 40 mg/dL), hemolysis (Hb < 0.311 mmol/L or < 0.500 g/dL), lipemia (triglycerides < 22.8 mmol/L or < 2000 mg/dL) and biotin (< 164 nmol/L or < 40 ng/mL).

Criterion: Recovery within ± 20 % 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 1200 IU/mL.

No high-dose hook effect was found with the Elecsys HBsAg II quant assay up to a concentration of 8.7 x 10^5 IU/mL when samples were analyzed according to instructions for use.

There is no indication for a significant loss in sensitivity or specificity with samples having elevated levels of albumin up to 14 g/dL.

No significant interfering effects of 22 commonly used therapeutic drugs could be detected (including lamivudine, peginterferon alpha-2a, entecavir, telbivudine and adefovir).

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

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

Make sure that in the Special Wash List (Screen → Utility → Special Wash → Immune) the Elecsys HBsAg II quant assay is combined with **all assays** performed on the analyzer - including the Elecsys HBsAg II quant assay itself:

From test	Step	To test	Step 0	Step 1	Step 2
HBsAg II quant	1	HBsAg II quant	x	x	x
HBsAg II quant	1	each other assay	x	x	x

If new tests are installed make sure that the Special Wash List is updated accordingly.

For the Elecsys Anti-HBs assay make sure that "Step 1" and "Step 2" are activated:

From test	Step	To test	Step 0	Step 1	Step 2
Anti-HBs	1	HBsAg II quant	-	x	x

The described additions to the Special Wash List have to be entered manually. 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.

Limits and ranges

Measuring range

Measuring range for pre-diluted samples:

5-13000 IU/mL for 100-fold diluted samples (Elecsys 2010 and **cobas e** 411 analyzers).

Values below the measuring range are reported as < 5 IU/mL.

Values above the measuring range are reported as > 13000 IU/mL.

20-52000 IU/mL for 400-fold diluted samples (**cobas e** 601, **cobas e** 602 and MODULAR ANALYTICS E170 analyzers).

Values below the measuring range are reported as < 20 IU/mL.

Values above the measuring range are reported as > 52000 IU/mL.

Measuring range for undiluted samples:

0.05-130 IU/mL (defined by the Limit of Detection (LoD) and the maximum of the master curve).

Values below the Limit of Detection are reported as < 0.05 IU/mL.

Values above the measuring range are reported as > 130 IU/mL.

Lower limits of measurement

Limit of Blank (LoB) and Limit of Detection (LoD)

Limit of Blank = 0.03 IU/mL

Limit of Detection = 0.05 IU/mL

The Limit of Blank and Limit of Detection were determined in accordance with the CLSI (Clinical and Laboratory Standards Institute) EP17-A requirements.

The Limit of Blank is the 95th percentile value from n ≥ 60 measurements of analyte-free samples over several independent series. The Limit of Blank corresponds to the concentration below which analyte-free samples are found with a probability of 95 %.

The Limit of Detection is determined based on the Limit of Blank and the standard deviation of low concentration samples. The Limit of Detection corresponds to the lowest analyte concentration which can be detected (value above the Limit of Blank with a probability of 95 %).

Dilution

Every sample has to be initially diluted with HBSAG-QN Dil HepB (mandatory dilution to be ordered on the respective platform).

The dilution factor for dilution by the Elecsys 2010 and **cobas e** 411 analyzers is 1:100.

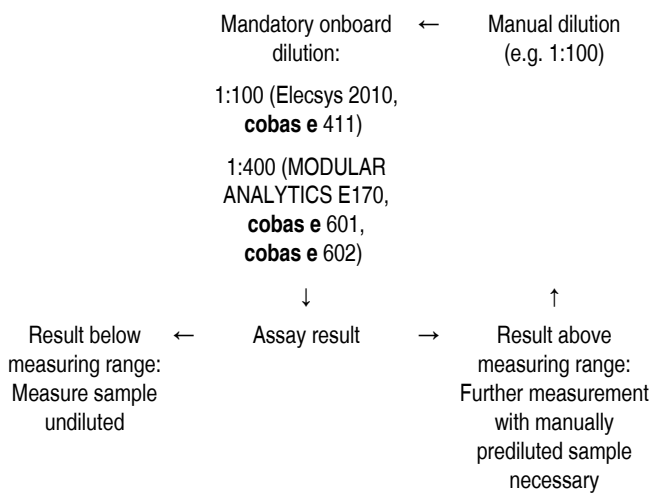
The dilution factor for dilution by the MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers is 1:400.

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.

Due to different dilutions performed on the different instrument platforms, minor deviations between the measurements on Elecsys 2010/ **cobas e** 411 analyzers and MODULAR ANALYTICS E170/ **cobas e** 601/ **cobas e** 602 analyzers might occur.

In highly concentrated patient samples, further manual dilution steps might be necessary to achieve results within the measuring range for pre-diluted samples. After manual dilution, multiply the result by the dilution factor chosen for the respective dilution step.

Test algorithm for samples:



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↓ Result within measuring range: Final result

↓ Result within measuring range: Final result

Initial onboard dilution is mandatory for every sample. Therefore every sample has to be run first with a dilution step ordered by the user and performed by the analyzer (1:100 on the Elecsys 2010 and **cobas e** 411 analyzers and 1:400 on the MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers).

If a result is found within 5-13000 IU/mL for 100-fold diluted samples or 20-52000 IU/mL for 400-fold diluted samples **no further dilution is necessary** and endpoint result is achieved.

If a result is found **below** the above mentioned **lower ranges**, the sample has to be run **undiluted** and should be found within 0.05-130 IU/mL.

If a result is found > 13000 IU/mL for 100-fold diluted samples or > 52000 IU/mL for 400-fold diluted samples **further manual dilution steps (e.g. additional 1:100 sample predilution prior to 1:100/1:400 instrument dilution to achieve a final 1:10000/1:40000 dilution)** are recommended until result is found within the measuring range.

Expected values

From 611 samples obtained from a multicenter-evaluation the following values have been reported:

IU/mL	MCE (n = 611)	% of total
< 1	17	2.78
1-< 10	20	3.27
10-< 100	35	5.73
100-< 1000	127	20.8
1000-< 10000	239	39.1
10000-< 100000	147	24.1
100000-< 1000000	26	4.26

The final result was determined from the first measurement in 70.0 % of the samples on the Elecsys 2010 and **cobas e** 411 analyzers (1:100 dilution) and 85.6 % of the samples on the MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers (1:400 dilution).

Each laboratory should investigate the transferability of the expected values to its own patient population and if necessary determine its own reference ranges.

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, samples and controls in a protocol (EP5-A2) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplication each for 21 days (n = 84). The following results were obtained:

Elecsys 2010 and **cobas e** 411 analyzers					
		Repeatability[b]		**Intermediate precision**[c]	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	3.07	0.056	1.8	0.17	5.6
Human serum 2	54.3	0.747	1.4	3.04	5.6
Human serum 3	6610	237	3.6	372	5.6
PreciControl HBsAg II 1	< 0.05	-	-	-	-
PreciControl HBsAg II 2	0.109	0.003	3.1	0.010	8.9

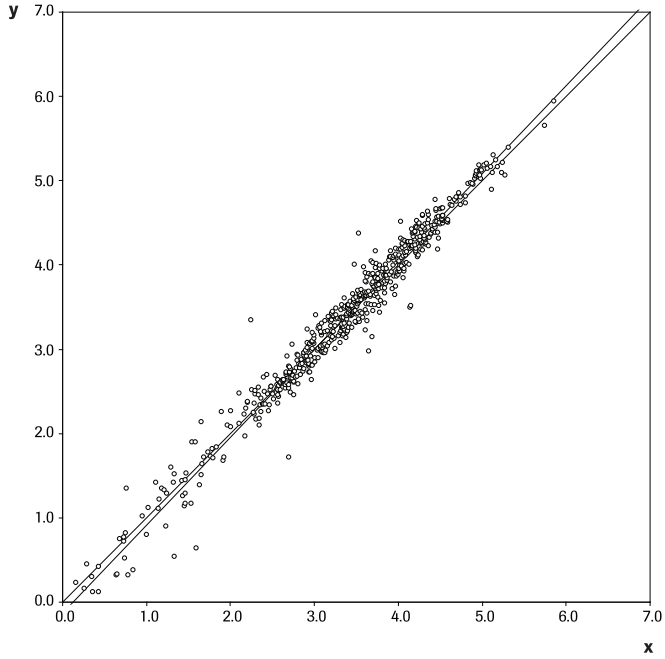
b) Repeatability = within-run precision

c) Intermediate precision = between-run

MODULAR ANALYTICS E170, **cobas e** 601 and **cobas e** 602 analyzers					
		Repeatability		**Intermediate precision**	
Sample	Mean IU/mL	SD IU/mL	CV %	SD IU/mL	CV %
Human serum 1	3.06	0.069	2.3	0.15	4.9
Human serum 2	55.5	1.48	2.7	3.64	6.6
Human serum 3	37300	1490	4.0	3590	9.6
PreciControl HBsAg II 1	< 0.05	-	-	-	-
PreciControl HBsAg II 2	0.130	0.007	5.6	0.010	7.6

Method comparison

A comparison of the Elecsys HBsAg II quant assay (y) with a commercially available HBsAg assay (x) using 611 serum samples of HBV infected patients across genotypes and phases of infection gave the following correlations (log10 IU/mL):



x: HBsAg comparison assay (log10 IU/mL)

y: Elecsys HBsAg II quant assay (log10 IU/mL)

Passing/Bablok[18]	Linear regression
y = 1.04x - 0.13	y = 1.03x - 0.10
τ = 0.900	r = 0.988

The sample concentrations were between approximately 0.11 and 873300 IU/mL.

Quantitation of potentially cross reactive samples

1285 samples containing potentially interfering substances were tested with the Elecsys HBsAg II quant assay comprising specimens:

- containing antibodies against HAV, HCV, HIV, HTLV, CMV, EBV, HSV, Rubella, Parvo virus, VZV, Toxoplasma gondii, Treponema pallidum
- containing autoantibodies (ANA, SLE), elevated titers of rheumatoid factor or HAMA antibodies
- positive for Mumps, Measles, Malaria
- after vaccination against HBV and influenza
- from patients with monoclonal gammopathy and multiple myeloma/lymphoma, patients undergoing dialysis or patients suffering from alcoholic liver disease

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- from pregnant women

No results were found ≥ 0.05 IU/mL.

Quantitation of HBV mutants

A total of 50 samples comprising different HBsAg mutations were tested with the Elecsys HBsAg II quant assay. Results of observed concentrations are displayed.

Mutant panel	Elecsys HBsAg II quant (IU/mL)[d)]
Native mutant panel (strains displaying amino acid substitutions either linked to vaccine resistance, resistance to therapy with human HB immunoglobulin or impaired HBsAg reactivity)	< 0.05 (n = 2) 0.05-324 (n = 17)
Recombinant mutant panel	> 0.05-6.9 (n = 31)

d) Observed concentrations with HBV mutants might differ compared to competitor assays and are a characteristic of the individual assays.

Seroconversion panels

18 seroconversion panels were analyzed with the Elecsys HBsAg II quant assay. In all panels the Elecsys HBsAg II quant assay shows a significant increase in concentration upon seroconversion correlated to the shift as it is detectable in qualitative screening assays. Observed concentrations ranged from < LoD for negative samples draws, and 0.058-92300 IU/mL for conversion samples (confirmed positives).

References

1 Gerlich W. Viral Hepatitis. Section 2, Churchil Livingstone, Ed. Zuckermann AJ, Thomas HC, 1993:83-113.

2 Hollinger FB. Hepatitis B virus. In Fields BN, Knipe DM (eds). Virology ed. 2 New York Raven Press 1990;2:2171-2236.

3 Coursaget P, Yvonnet B, Bourdil C, et al. HBsAG positive reactivity in man not due to hepatitis B virus. Lancet 1987 Dec 12;2(8572):1354-1358.

4 Shiels MT, Taswell HF, Czaja AJ, et al. Frequency and significance of concurrent hepatitis B surface antigen and antibody in acute and chronic hepatitis B. Gastroenterology 1987 Oct;93(4):675-680.

5 European Association for the Study of the Liver. EASL Clinical Practice Guidelines: Management of chronic hepatitis B. J Hepatol 2009;50:227-242.

6 Moucari R, Mackiewicz V, Lada O, et al. Early serum HBsAg drop: A strong predictor of sustained virological response to pegylated interferon alfa-2a in HBeAg-negative patients. Hepatology 2009;49:1151-1157.

7 Marcellin P, Bonino F, Lau GK, et al. Sustained response of hepatitis B e antigen-negative patients 3 years after treatment with peginterferon alpha-2a. Gastroenterology 2009;136(7):2169-2179.

8 Perillo R. Hepatitis B Surface Antigen Quantification as a Current-Day Paradox: Obtaining the Gold in the Face of Diminishing Returns. Hepatology 2009, Vol. 49, No. 4.

9 Brunetto MR, Moriconi F, Bonino F, et al. Hepatitis B Virus Surface Antigen Levels: A Guide to Sustained Response to peginterferon alfa-2a in HBeAg-Negative Chronic Hepatitis B. Hepatology 2009;49:1141-1150.

10 Chan H LY, Wong V WS., Tse A ML, et al. Serum Hepatitis B Surface Antigen Quantitation Can Reflect Hepatitis B Virus in the Liver and Predict Treatment Response. Clin Gastroenterol Hepatol 2007;5:1462-1468.

11 Gish RG, Lau D TY, Schmid P, et al. A Pilot Study of Extended Duration Peginterferon Alfa-2a for Patients With Hepatitis B e Antigen-Negative Chronic Hepatitis B. Am J Gastroenterol 2007;102:2718-2723.

12 Wursthorn K, Jung M, Goodman Z, et al. Serum HBsAg kinetics in HBeAg-positive patients during 3 years telbivudine treatment in chronic hepatitis B. Poster No. 188, 20th Asian Pacific Association for the Study of the Liver conference, 2010, Beijng, China.

13 Nguyen T, Thompson AJ, Bowden S, et al. Hepatitis B surface antigen levels during the natural history of chronic hepatitis B: a perspective on Asia. J Hepatol 2010 Apr;52(4):508-513.

14 Brunetto MR, Marcellin P, Bonino F, et al. HBsAg Decline in HBeAg-Negative Patients Treated With Peginterferon Alfa-2A is Associated with Sustained Response up to 5 Years Post-Treatment: Patients with Continuous HBsAg Decline Starting Before Week 24 Achieve Highest Rates of Response. Hepatology 2009; Volume 50 Issue S4, Pages 1-1246, poster 452.

15 Martinot-Peignoux M., Moucari R, Leclere L, et al. Quantitative HBsAg: A New Specific Marker for the Diagnosis of HBsAg Inactive Carriage. J Hepatol 2010;vol. 52:183-317, No. 725.

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

17 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.

18 Bablok W, Passing H, Bender R, et al. A general regression procedure for method transformation. Application of linear regression procedures for method comparison studies in clinical chemistry, Part III. J Clin Chem Clin Biochem 1988 Nov;26(11):783-790.

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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Roche