Elecys Anti-HBs II

Intended use

Immunoassay for the in vitro quantitative determination of human antibodies to the hepatitis B surface antigen (HBsAg) in human serum and plasma. Anti-HBs assays are used within the scope of hepatitis B vaccination to check the necessity and success of vaccination. In addition, anti-HBs assays are used to monitor the course of disease following acute hepatitis B infection. This test is not intended for diagnosis. The electrochemiluminescence immunoassay “ECLIA” is intended for use on the cobas e 801 immunoassay analyzer.

Note: Please note that the catalogue number appearing on the package insert retains only the first 8 digits of the licensed 11-digit Catalogue Number: 07026854190 for the Elecsys Anti-HBs II assay. The last 3 digits -190 have been replaced by -119 for logistical purposes.

Summary

Anti-HBs is a specific (generally IgG) antibody that is directed against the hepatitis B surface antigen (HBsAg). Anti-HBs can be detected several weeks after the disappearance of hepatitis B surface antigen. 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.

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

The Elecsys Anti-HBs II assay uses a mixture of purified antigens from human serum (HBsAg subtype ad), and recombinant HBsAg subtype ay from CHO (Chinese Hamster Ovary) cells.

Test principle

Sandwich principle. Total duration of assay: 18 minutes.

1. 1st incubation: Anti-HBs in the sample (24 μL), biotinylated HBsAg (ad/ay), and HBsAg (ad/ay) labeled with a ruthenium complex react to form a sandwich complex.

2. 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 II M.

Results are determined via a calibration curve which is instrument-specifically generated by Z-point calibration and a master curve provided via the cobas link.

Reagents – working solutions

The cobas e pack (M, R1, R2) is labeled as AHBS 2.

M Streptavidin-coated microparticles, 1 bottle, 13.2 mL:

R1 HBsAg-biotin, 1 bottle, 16.7 mL:

R2 HBsAg-Ru(bpy) 3+ , 1 bottle, 15.8 mL:

Reagents handling

The reagents (M, R1, R2) in the kit are ready-for-use and are supplied in cobas e packs.

Calibrators

The calibrators are supplied ready-for-use in bottles compatible with the system.

Storage and stability

Store at 2-8 °C.

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-propanesulphonate

EUH 208 May produce an allergic reaction.

Product safety labeling primarily follows EU GHS guidance.

Preparation and use of the calibrators and controls.

Stability of the AHBS 2 Calibrator 1, 1 bottle of 1.3 mL:

Anti-HBs (human) in human serum; preservative.

Stability of the AHBS 2 Calibrator 2, 1 bottle of 1.3 mL:

Anti-HBs (human) in human serum; preservative.

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Stability of the AHBS 2 Calibrator 2, 1 bottle of 1.3 mL:

Anti-HBs (human) in human serum; preservative.
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.

K₂-EDTA and K₃-EDTA plasma.

Criterion: Slope 1.00 ± 0.15 + intercept 0 ± 2 IU/L + bias at 10 IU/L ≤ 30 %.

Stable for 3 days at 20-25 °C, 6 days at 2-8 °C, 3 months at -20 °C(± 5 °C).

The samples may be frozen 5 times.

For plasma treated with lithium heparin, lithium heparin with gel or sodium heparin, the values found were on average up to 20 % lower than those obtained in serum. For plasma treated with sodium citrate, the values found were on average up to 30 % lower than those obtained with serum.

The sample types listed were tested with a selection of sample collection tubes or systems 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 thawed samples before performing the assay.

Do not use heat-inactivated samples.

Do not use samples and controls stabilized with azide.

Due to possible evaporation effects, samples and calibrators on the analyzers are at 20-25 °C prior to measurement.

Endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and compounds on assay performance was tested.

Respective recommendations are given by national or regional guidelines.

Materials required (but not provided)

See “Reagents – working solutions” section for reagents.

- 2 x 6 bottle labels

Materials provided

Accessories for the cobas e 801 analyzer:

- 07299001190, Diluent Universal, 45.2 mL sample diluent
- 06908799190, Procell II M, 2 x 2 L system solution
- 04880293190, CleanCell M, 2 x 2 L measuring cell cleaning solution
- 07485049001, Reservoir Cups, 8 cups to supply Procell II M and CleanCell M
- 06908853190, PreClean II M, 2 x 2 L wash solution
- 05694302001, Assay Tip/Assay Cup tray, 6 magazines x 6 magazine stacks x 105 assay tips and 105 assay cups, 3 wasteliners
- 07485425001, Liquid Flow Cleaning Cup, 2 adaptor cups to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning Detection Unit
- 07485433001, PreWash Liquid Flow Cleaning Cup, 1 adaptor cup to supply ISE Cleaning Solution/Elecsys SysClean for Liquid Flow Cleaning PreWash Unit
- 011298500316, 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. Place the cooled (stored at 2-8 °C) cobas e pack on the reagent manager. Avoid foam formation. The system automatically regulates the temperature of the reagents and the opening/closing of the cobas e pack.

Calibrators:

Place the calibrators in the sample zone.

Read in all the information necessary for calibrating the assay.

Calibration

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

The predefined master curve is adapted to the analyzer using AHBS 2 Cal1 and AHBS 2 Cal2.

Calibration frequency: Calibration must be performed once per reagent lot using AHBS 2 Cal1, AHBS 2 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 12 weeks when using the same reagent lot
- after 28 days when using the same cobas e pack on the analyzer
- as required: e.g. quality control findings with PreciControl Anti-HBs outside the defined limits

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 cobas e pack, 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 of each sample in IU/L.

Interpretation of the results

<table>
<thead>
<tr>
<th>Numeric result</th>
<th>Result message</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 10 IU/L</td>
<td>Non-reactive</td>
<td>Negative for anti-HBs</td>
</tr>
<tr>
<td>≥ 10 IU/L</td>
<td>Reactive</td>
<td>Positive for anti-HBs</td>
</tr>
</tbody>
</table>

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). 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 method must be confirmed by parallel measurements by both methods. Vaccination strategies in certain risk groups are based on the measured anti-HBs concentration. Respective recommendations are given by national or regional guidelines.

Limitations - interference

The effect of the following endogenous substances and pharmaceutical compounds on assay performance was tested. Interferences were tested up to the listed concentrations and no impact on results was observed.

Endogenous substances

<table>
<thead>
<tr>
<th>Compound</th>
<th>Concentration tested</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bilirubin</td>
<td>≤ 51.3 µmol/L or ≤ 30 mg/dL</td>
</tr>
<tr>
<td>Hemoglobin</td>
<td>≤ 0.621 mmol/L or ≤ 1000 mg/dL</td>
</tr>
<tr>
<td>Intralipid</td>
<td>≤ 1500 mg/dL</td>
</tr>
<tr>
<td>Biotin</td>
<td>≤ 41 nmol/L or ≤ 10 ng/mL</td>
</tr>
<tr>
<td>Rheumatoid factors</td>
<td>≤ 1200 IU/mL</td>
</tr>
<tr>
<td>Albumin</td>
<td>≤ 7.0 g/dL</td>
</tr>
<tr>
<td>IgG</td>
<td>≤ 7.0 g/dL</td>
</tr>
<tr>
<td>IgA</td>
<td>≤ 1.6 g/dL</td>
</tr>
<tr>
<td>IgM</td>
<td>≤ 1.0 g/dL</td>
</tr>
</tbody>
</table>
Elecsys Anti-HBs II

Criterion: Recovery for samples from Limit of Detection to 10 IU/L: ≤ ± 2 IU/L, and samples > 10 IU/L: ≤ ± 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.

Pharmaceutical substances
In vitro tests were performed on 16 commonly used pharmaceuticals. No interference with the assay was found.

In addition, the following special drugs used in hepatitis B therapy were tested. No interference with the assay was found.

Special drugs

<table>
<thead>
<tr>
<th>Drug</th>
<th>Concentration tested mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peginterferon alfa-2a</td>
<td>≤ 0.18</td>
</tr>
<tr>
<td>Peginterferon alfa-2b</td>
<td>≤ 1.6</td>
</tr>
<tr>
<td>Lamivudine</td>
<td>≤ 300</td>
</tr>
<tr>
<td>Adefovir</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Entecavir</td>
<td>≤ 10</td>
</tr>
<tr>
<td>Tenofovir</td>
<td>≤ 600</td>
</tr>
<tr>
<td>Telbivudine</td>
<td>≤ 245</td>
</tr>
</tbody>
</table>

Due to high-dose hook effect\(^d\), results from anti-HBs concentrations of > 20000 IU/L may be found below the upper limit of the measuring range of 1000 IU/L. In rare cases, a high-dose hook effect from anti HBs concentrations of < 20000 IU/L cannot be excluded. Therefore in case of any unexpected low result the sample should be diluted 1:100 (refer to chapter "Dilution") and tested again.

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.

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
2-1000 IU/L (defined by the Limit of Detection and the maximum of the master curve). Values below the Limit of Detection are reported as < 2 IU/L.

Values above the measuring range are reported as > 1000 IU/L (or up to 100000 IU/L for 100-fold diluted samples).

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 analyzer 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 analyzer, the 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 heterogeneous. In some isolated cases, this may lead to non-linear dilution behavior.

Specific performance data

Representative performance data on the analyzer is given below. Results obtained in individual laboratories may differ.

Precision

Precision was determined using Elecsys reagents, samples and controls in a protocol (EP05-A3) of the CLSI (Clinical and Laboratory Standards Institute): 2 runs per day in duplicate each for 21 days (n = 84). The following results were obtained:

<table>
<thead>
<tr>
<th>Cobas e 801 analyzer</th>
<th>Repeatability</th>
<th>Intermediate precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample</td>
<td>Mean IU/L</td>
<td>SD IU/L</td>
</tr>
<tr>
<td>Human serum 1</td>
<td>4.33</td>
<td>0.224</td>
</tr>
<tr>
<td>Human serum 2</td>
<td>12.0</td>
<td>0.237</td>
</tr>
<tr>
<td>Human serum 3</td>
<td>475</td>
<td>6.81</td>
</tr>
<tr>
<td>PC Anti-HBs 1</td>
<td>&lt; 2.00</td>
<td>-</td>
</tr>
<tr>
<td>PC Anti-HBs 2</td>
<td>83.8</td>
<td>1.08</td>
</tr>
</tbody>
</table>

d) Repeatability = within-run precision

e) Intermediate precision = between-run precision

f) PC = PreciControl

Analytical specificity

No cross-reactions with HAV, HCV, HEV, CMV, EBV, HIV, Rubella, Toxoplasma gondii, Treponema pallidum, rheumatoid arthritis, autoimmune response or alcoholic liver disease were observed.

Measurements were performed on each of the pathogens listed above using ≥ 8 serum or plasma samples which were positive for antibodies to the above-mentioned pathogens.

Relative sensitivity

Performance of the Elecsys Anti-HBs II assay has been assessed by testing a total of 669 samples at two different study sites. 296 samples from vaccinated persons and 373 samples from patients recovered from a hepatitis B infection have been measured with the Elecsys Anti-HBs II assay and another commercially available fully automated anti-HBs assay.

Discrepant samples were tested with additional anti-HBs assays to achieve a consensus.

Relative specificity

Performance of the Elecsys Anti-HBs II assay has been assessed by testing 2673 samples from blood donors negative for anti-HBs at two different study sites and 1623 anti-HBs negative samples from laboratory routine at three different study sites. Discrepant samples were tested with additional anti-HBs assays to achieve a consensus.

References


Elecsys Anti-HBs II


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:

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTENT</td>
<td>Contents of kit</td>
</tr>
<tr>
<td>SYSTEM</td>
<td>Analyzers/Instruments on which reagents can be used</td>
</tr>
<tr>
<td>REAGENT</td>
<td>Reagent</td>
</tr>
<tr>
<td>CALIBRATOR</td>
<td>Calibrator</td>
</tr>
<tr>
<td>GTIN</td>
<td>Volume after reconstitution or mixing</td>
</tr>
<tr>
<td>STIN</td>
<td>Global Trade Item Number</td>
</tr>
</tbody>
</table>

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