Enzygnost® Anti-HBs II

Intended Use

Enzyme immunoassay for the qualitative detection and quantitative determination of specific antibodies to the hepatitis B surface antigen (HBsAg) in human serum and plasma.

The enzyme immunoassay can be processed using the ELISA processors, BEP® III System, BEP® 2000 System or the BEP 2000 Advance® System. A non-automated processing of the test is also possible.

Summary and Explanation

Like the hepatitis B surface antigen (HBsAg), the anti-HBs antibody directed against the surface protein is an important parameter for the diagnosis of infection with the hepatitis B virus (HBV)\(^1\). \(^2\).

During the incubation period and in the acute phase of an HBV infection, antibodies to HBsAg are undetectable. In 90% of all cases these anti-HBs antibodies, which provide immunity, do not occur until late in convalescence approximately 3 to 4 months after the onset of the disease, when circulating HBsAg can no longer be detected\(^3\).\(^4\).

This seroconversion, i.e. the transition from anti-HBs negative to positive, represents a very reliable parameter for diagnosing a past HBV infection, especially since approximately 10% of acutely infected patients in whom no HBsAg can be detected in the early phase later become positive for anti-HBs. The anti-HBs test is therefore also suitable for the diagnosis for subclinical HBV infections\(^3\).\(^4\).

As a positive result for anti-HBs is indicative of past exposure to this antigen - either by HBV infection or by vaccination, the most important applications of the anti-HBs test are as follows:

a. Assessment of convalescence in HBV-infected patients (progress monitoring).

b. Serological investigations in the context of vaccination programs (screening and immunization check-ups).

c. Epidemiological studies.

Besides the qualitative detection of anti-HBs, the quantitative evaluation of anti-HBs has gained its own relevance with regard to the aspect of active immunization. According to a recommendation of the WHO, a person vaccinated with a hepatitis vaccine can be assumed to be protected against the infection if an anti-HBs concentration of > 10 IU/L can be detected in the serum or plasma. Booster vaccinations in time are recommended to ensure that the values are not below this limit\(^5\).\(^6\).

Patients found to have anti-HBs values < 100 IU/L after the completion of basic immunization require booster vaccination within one year (Recommendations of the Standing Committee on Immunization of the Robert Koch Institute in Germany, October 1995).

The necessity of a quantitative assessment is furthermore underlined by the fact that the duration of immunity is proportional to the attained levels of anti-HBs following vaccination.

Principle of the Method

Enzygnost® Anti-HBs II is a one-step assay based on the sandwich principle. Inactivated HBsAg of human origin containing subtypes ad and ay is used as solid phase and as conjugate antigen.
Peroxidase-labeled HBsAg binds to the HBs-specific antibodies contained in the sample. These bind to the HBsAg bound to the surface of the microtitration plate (antigen sandwich).

The enzyme portion of the conjugate causes the Chromogen Working Solution to turn blue. This reaction is stopped by the addition of Stopping Solution POD, which causes a color change to yellow. The intensity of the yellow color produced is proportional to the activity of specific antibody contained in the sample. Quantification in International Units is performed by calculation using the α-method.

Reagents

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Materials provided</th>
</tr>
</thead>
<tbody>
<tr>
<td>MTP</td>
<td>Enzygnost® Anti-HBs II 1 x 96 10 x 96</td>
</tr>
<tr>
<td>CONJUGATE</td>
<td>Anti-HBs II test plate 1 pc. 10 pcs.</td>
</tr>
<tr>
<td>REFER P</td>
<td>Anti-HBs II Conj 1 x 6 mL 10 x 6 mL</td>
</tr>
<tr>
<td>REFER N</td>
<td>Anti-HBs II REF P 1 x 1.5 mL 3 x 1.5 mL</td>
</tr>
<tr>
<td>WASH POD</td>
<td>Anti-HBs II Neg 2 x 14 mL 5 x 14 mL</td>
</tr>
<tr>
<td>SUBSTRATE TMB</td>
<td>Washing Solution POD* 1 x 100 mL 2 x 100 mL</td>
</tr>
<tr>
<td>CHROMOGEN TMB</td>
<td>Buffer/Substrate TMB* 1 x 30 mL 4 x 30 mL</td>
</tr>
<tr>
<td>STOP POD</td>
<td>Chromogen TMB* 1 x 3 mL 4 x 3 mL</td>
</tr>
<tr>
<td>EMPTY VIAL CHROM SOL</td>
<td>Stopping Solution POD* 1 x 100 mL 2 x 100 mL</td>
</tr>
<tr>
<td></td>
<td>Empty bottle for Working Chromogen Solution* 1 pc. 1 pc.</td>
</tr>
<tr>
<td></td>
<td>Adhesive foils 6 pcs. 24 pcs.</td>
</tr>
<tr>
<td></td>
<td>Polyethylene bag 1 pc. 1 pc.</td>
</tr>
<tr>
<td></td>
<td>Barcode table of values 1 pc. 1 pc.</td>
</tr>
<tr>
<td></td>
<td>Instructions for Use 1 pc. 1 pc.</td>
</tr>
</tbody>
</table>

Further kit: 100 x 96

* These components are also included in the kit Supplementary Reagents for Enzygnost®/TMB (REF OUVP).

The test plate, the conjugate as well as Anti-HBs II REF P and Anti-HBs II Neg must be used in the given combination of 6-digit lot numbers printed on the package, respectively stated in the enclosed barcode table of values. The same applies to the reagents Chromogen TMB and Buffer/Substrate TMB.

Composition

**Enzygnost® Anti-HBs II test plate:** microtitration plate coated with inactivated HBsAg (subtypes ad and ay) isolated from human blood

**Anti-HBs II Conj:** inactivated HBsAg from human blood (subtypes ad and ay), peroxidase (POD)-conjugated in TRIS/HCl buffer with sodium chloride, ready-for-use

Preservative: phenol (≤ 1 g/L)

**Anti-HBs II REF P:** anti-HBs positive human serum, nominal absorbance: ≥ 0.7

Preservatives:
- amphotericin (≤ 5 mg/L)
- gentamicin (≤ 100 mg/L)

**Anti-HBs II Neg:** anti-HBs negative human serum, nominal absorbance: ≤ 0.120

Preservatives:
- amphotericin (≤ 5 mg/L)
- gentamicin (≤ 100 mg/L)

**Washing Solution POD (concentrate):** phosphate buffer containing Tween

Preservative: phenol (≤ 1 g/L)

**Buffer/Substrate TMB:** hydrogen peroxide in acetate buffer

Preservative: n-butanol (≤ 1 %)
**Chromogen TMB**: tetramethylbenzidine dihydrochloride

**Stopping Solution POD**: sulfuric acid (0.25 mol/L)

**Warnings and Precautions**

For **in-vitro** diagnostic use only.

The test was developed for testing individual samples, not for pooled samples.

**Warning! [STOP POD]**

_H290, H314_: May be corrosive to metals. Causes severe skin burns and eye damage.


Immediately call a POISON CENTER or doctor/physician. IF SWALLOWED: Rinse mouth.

Do NOT induce vomiting. IF ON SKIN (or hair): Remove/Take off immediately all contaminated clothing. Rinse skin with water/shower. IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing. Absorb spillage to prevent material damage. Dispose of contents and container in accordance with all local, regional, and national regulations.

**CAUTION! POTENTIAL BIOHAZARD**

Each donor or donor unit was tested and found to be negative for human immunodeficiency virus (HIV) 1 and 2, hepatitis B virus (HBV) and hepatitis C virus (HCV) using either tests found to be in conformance with the In Vitro Diagnostic Directive in the EU or FDA approved tests. Because no known test can offer complete assurance of the absence of infectious agents, all human derived products should be handled with appropriate caution.

Safety data sheets (MSDS/SDS) available on www.siemens.com/diagnostics

The HBsAg used for manufacturing the test plates and POD conjugate was isolated to a high degree of purity from HBsAg-positive human plasmas, which were found to be Anti-HCV, Anti-HIV1 and Anti-HIV2 negative, and subjected to a recognized inactivation process.

It is advisable to wear protective gloves throughout the entire test procedure. Please follow the recommendations of the manufacturer concerning the compatibility between gloves and exposed materials.

For disposal, it is recommended that solid infectious materials should be autoclaved for at least one hour at 121 °C. All aspirated solutions must be collected in two receptacles connected in series. Both should contain a disinfectant suitable for inactivating human pathogens. The concentrations and times specified by the manufacturer must be observed.

Buffer/Substrate TMB, Chromogen Working Solution and Stopping Solution POD must not be allowed to come into contact with heavy metal ions or oxidizing substances (do not use pipettes with metal parts which are in direct contact with the liquid). The substrate reaction steps must not be performed in the vicinity of disinfectants containing hypochlorite. If the Chromogen Working Solution has spontaneously developed a blue color before transferal into the test plate, this indicates that the solution is contaminated; in such cases, prepare a fresh solution in a clean container. Skin contact with the aforementioned solutions is to be avoided.

**Preparation of the Reagents**

Bring all reagents and test samples to 15 to 25 °C before starting with the test. Do **not** remove the foil pouch from the test plates during this step. Before starting the test processing remove not required strips from the holder and store these in the enclosed polyethylene bag for later use (see Table 1). If reagents or reagent working solutions need to be mixed, avoid foam formation.
For each test plate, dilute **20 mL of Washing Solution POD** with distilled or deionized water to **400 mL**.

For each test plate, dilute **1 mL of Chromogen TMB with 10 mL of Buffer/Substrate TMB** (Chromogen Working Solution) using the supplied empty plastic bottle. Store protected from light. After use, carefully rinse the bottle with distilled or deionized water. For technical reasons (overfill) it is not permissible to pour together the full contents of the Chromogen TMB vial and the full contents of the Buffer/Substrate TMB vial.

**Sample Preparation**

Enzygnost® Anti-HBs II covers a minimum measuring range of between 10 IU/L and 100 IU/L. Samples with anticipated Anti-HBs concentrations of ≥ 100 IU/L can be diluted as shown below using Anti-HBs II Neg included in the test kit:

- **Up to 1,000 IU/L**: dilution 1+9 (e.g. 20 µL sample + 180 µL Anti-HBs II Neg).
- **Up to 10,000 IU/L**: dilution 1+99 (e.g. 20 µL sample (1+9) + 180 µL Anti-HBs II Neg).
- **Up to 100,000 IU/L**: dilution 1+999 (e.g. 20 µL sample (1+99) + 180 µL Anti-HBs II Neg).

**Storage and Stability**

Stored unopened at the stated temperature, all components of the Enzygnost® Anti-HBs II kit may be used up to the expiry dates given on the labels.

For complete stability and storage data of the opened, respectively diluted reagents, see Table 1 in the Appendix.

**Equipment Required**

- **BEP® III**: for automated processing of the test after dispensing the samples as well as for evaluation
- **BEP® 2000/BEP 2000 Advance®**: for fully automated processing and evaluation of the test
- **Pipettes**: piston-type pipettes with fixed or variable volumes, or single- and multi-channel pipettes with adjustable volumes

The following items are required additionally if the test is not processed automatically:

- **Incubator**: covered thermostatic water bath (37 ±1 °C) or a similar incubation method
- **Washing device**: microtitration plate washer
- **Photometer**: photometer suitable for microtitration plates, measuring wavelength of 450 nm, reference wavelength of 650 nm (between 615 nm and 690 nm as appropriate)

For quantitative test evaluation: pocket calculator with exponential and logarithm function

All the equipment used in the test must have been validated.

**Specimens**

Suitable specimens are individual samples (human sera or EDTA/heparinized/citrated plasma) obtained by standard laboratory techniques. Heparinized and citrated plasma specimens should be stored for no more than 3 days at 2 to 8 °C, whereas serum and EDTA plasma can be used up to 8 days under these storage conditions. If samples are to be stored for a longer period of time, they must be frozen.

**Procedure**

**Non-automated Test Procedure**

1. **Assay scheme**: The necessary number of test plate wells is given by the number of samples plus the number of determinations \( n = 6 \) for Anti-HBs II Neg and Anti-HBs II REF P.

2. **Dispense conjugate**: Add 25 µL of the Anti-HBs II Conj to each well. Pipette the controls and samples immediately after completing the conjugate dispensing step.
3. **Dispense samples**: Dispense 100 µL of Anti-HBs II Neg into each of 4 wells (A1-D1), 100 µL Anti-HBs II REF P into one well (E1) and 100 µL sample into each of the subsequent wells. At the end of the series respectively test plate fill one further well with 100 µL Anti-HBs REF P.

**Important:** It is NOT permitted to first pipette the Anti-HBs II REF P into the wells at the start and end of the sample series, and then put the samples in-between.

Each sample must be pipetted with its own pipette tip. The pipetting steps must be completed within 30 minutes per test plate. After completing the pipetting steps, seal the test plate with foil and place immediately into the incubator.

4. **Incubate**: Incubate for 60 ±2 minutes at 37 ±1 °C, then proceed immediately to the wash step.

5. **Wash**: Remove foil and aspirate all wells. Fill each well with approx. 300 µL diluted Washing Solution POD, aspirate the plate and repeat wash cycle three times. After completing the wash cycles, proceed immediately to the next reagent dispensing step (otherwise the wells may dry out).

6. **Dispense substrate**: Pipette 100 µL of the Chromogen Working Solution into each well and seal the microtitration plate with fresh foil.

7. **Incubate substrate**: Immediately after the substrate dispensing step, incubate at 15 to 25 °C for 30 ±2 minutes protected from light.

8. **Stop reaction**: Remove the foil. Add 100 µL of Stopping Solution POD to each well, keeping to the same timing as during the substrate dispensing.

9. **Measure**: Read the test plate at 450 nm within one hour. The recommended reference wavelength is 650 nm, or where appropriate, between 615 and 690 nm.

**Procedure for the BEP® III System**

When using the BEP® III, the test plates must be prepared up to the sample dispensing step (steps 1 to 3 in the section "Non-automated Test Procedure"). Ensure that partially loaded test plates are supplemented with water-filled strips to at least half plates (6 test strips).

Immediately afterwards place the uncovered test plates (i.e. not covered with foil) into the BEP® III. All subsequent processing steps are performed fully automatically by the instrument (see BEP® III Instruction Manual).

The settings for the incubation times in the BEP® III software may differ from the times in the section "Non‑automated Test Procedure" for technical reasons (system speed) but have been validated for Enzygnost® on the BEP® III.

**Procedure for the BEP® 2000 System**

The sample dispensing steps and subsequent processing of the test are performed fully automatically by the analyzer (see BEP® 2000 Instruction Manual). When doing so, ensure that partially loaded test plates are supplemented with water-filled strips to at least half plates (6 test strips).

Sample processing with the BEP® 2000 System may differ from the information given under "Non‑automated Test Procedure", but has been validated for Enzygnost® on the BEP® 2000.

**Internal Quality Control**

**Validation Criteria**

To evaluate the test the following criteria must be fulfilled:

1. Anti-HBs II Neg: \(-0.010 \leq A \leq 0.120\)
2. Anti-HBs II REF P: \(A \geq 0.7\)

**One** value for Anti-HBs II Neg that lies outside the specification can be ignored.

Both absorbance values of Anti-HBs II REF P, must fulfill the specification.

If these conditions are not met, the test is not valid for evaluation. In this case, the software of BEP® III and BEP® 2000 will give the notice "Invalid Test". The measurements must be repeated after investigating the cause.
For the quantitative test the following validation criteria apply:

Anti-HBs II REF P:
The individual absorbance values must lie within the lower and upper margins listed in the respective barcode table of values:
lower margin < $A_{\text{Anti-HBs II REF P}}$ < upper margin

In addition, the individual values (Anti-HBs II REF P at the start and end of a series of measurements or test plate) must not differ by more than ±20% from the mean value calculated from these values.

If these conditions are not met, the test can only be evaluated qualitatively.

Results

The evaluations are performed automatically if using the BEP® III System and BEP® 2000 System. Please consult the relevant Instruction Manual. The following sections must be taken into account when performing measurements without software support.

Qualitative Evaluation

To calculate the cut-off, use the mean of the valid absorbance values of Anti-HBs II Neg and add a value of 0.08:

$$\bar{A}_{\text{Anti-HBs II Neg}} + 0.08 = \text{cut-off}$$

Based on the criteria of the test the samples are classified as follows:

1. Anti-HBs negative $A_{\text{Sample}} < \text{cut-off}$
2. Anti-HBs positive $A_{\text{Sample}} \geq \text{cut-off}$

Quantitative Evaluation with the Aid of the α-Method

Measurement Correction

For achieving an optimal reproducibility of the results, the measurements require correction.

First, calculate the mean absorbance value for Anti-HBs II REF P. Then divide the nominal value, provided in the barcode table of values, by the calculated mean absorbance value of Anti-HBs II REF P:

Correction factor = $\frac{\text{A nominal value}}{\text{Mean } A_{\text{value}}_{\text{Anti-HBs II REF P}}}$

The absorbance values of those test samples determined in the series must now be multiplied by this correction factor.

If processing several test plates, the correction factor must be calculated and used for each individual test plate.

Calculation of the Results

Samples with an antibody activity higher than the cut-off value can be quantitatively analyzed using the α-method.

Do not use for calculation:
- Readings (A) corrected < cut-off
- Readings (A) uncorrected ≥ 2.5

The calculation is performed according to the following formula:

$$\log_{10} \text{mIU/L} = \alpha \times A^\beta$$

The lot-specific values for the constants α and β can be taken from the enclosed barcode table of values. To convert to IU/L the result must be divided by 1000.

Samples with an absorbance value (uncorrected) ≥ 2.5 must be tested in a dilution of e.g. 1+9 for a valid evaluation. Then, the result (not the reading) must be multiplied by 10.

The antibody activity values in International Units are traceable to “Human Anti-Hepatitis B Immunoglobulin” (2nd International Standard 2008, NIBSC code 07/164) of the WHO. 

7
Limitations of the Procedure

1. Anticoagulants such as heparin, EDTA and citrate do not interfere with the test.
2. Samples that are lipemic, hemolytic, icteric or that contain rheumatoid factors do not impair the test results.
3. In the case of samples from pregnant women, no interference with the test result has been observed.
4. Samples with the following substances which have the potential to interfere with the result have been checked with the test: ANA as well as antibodies against HCV, HAV, EBV and CMV. With the samples used, no interference was observed with the test result.
5. No interferences have been observed with heat-treated samples (30 minutes, 56 °C).
6. Incompletely coagulated sera and sample material containing sodium azide or microbial contamination should not be used. Any particles in the sample (e.g., fibrin clots) should be removed before the test.
7. If thawed samples are used, ensure that the material is thoroughly homogenized.
8. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
9. The references were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
10. The test plate should remain fixed during incubation (e.g., placed on a secured floatation aid, or in a non-circulating water bath); the wells of the plate must be in contact with the temperature-controlled water. If stabilizers are used to prevent microbial contamination of the water, care must be taken that neither the surface of the test plate nor the wells come into contact with the liquid since such contamination can lead to unspecific reactions.
11. Siemens Healthcare Diagnostics has validated use of these reagents on various analyzers to optimize product performance and meet product specifications. User defined modifications are not supported by Siemens as they may affect performance of the system and assay results. It is the responsibility of the user to validate modifications to these Instructions for Use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.
12. Results of this test should always be interpreted in conjunction with the patient’s medical history, clinical presentation and other findings.

Specific Performance Characteristics

Sensitivity and Specificity

The diagnostic sensitivity was established by investigating 755 anti-HBs positive samples, and the sensitivity result was 99.1 % (see Table 2). This result demonstrated that the sensitivity of Enzygnost® Anti-HBs II is equal to that of comparable tests.

Analytical sensitivity was determined using a series of dilutions of the WHO reference preparation as < 8 IU/L at the cut-off value.

For establishing the specificity of the test, a total of 824 anti-HBs negative blood donor samples were investigated and the result was 99.8 %.

In relation to sample population, test procedure, and other factors different values may be obtained, which however have to be in accordance with the Common Technical Specifications (CTS).

Precision

The results are summarized in Table 3.

Note

The values cited for performance characteristics of the assay represent typical results and are not to be regarded as specifications for Enzygnost® Anti-HBs II.
Bibliography


Definition of Symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>×</td>
<td>Do not reuse</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch Code</td>
</tr>
<tr>
<td>⚠️</td>
<td>Caution, consult accompanying documents</td>
</tr>
<tr>
<td>EC REP</td>
<td>Authorized representative in the European Community</td>
</tr>
<tr>
<td>🧪</td>
<td>Biological Risks</td>
</tr>
<tr>
<td>🌵</td>
<td>Temperature Limitation</td>
</tr>
<tr>
<td>🏗️</td>
<td>Non-sterile</td>
</tr>
<tr>
<td>CONTENTS</td>
<td>Contents</td>
</tr>
<tr>
<td>LEVEL</td>
<td>Level</td>
</tr>
<tr>
<td>YYYY-MM-DD</td>
<td>Use By</td>
</tr>
<tr>
<td>REF</td>
<td>Catalogue Number</td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
<tr>
<td>Contains sufficient for &lt;n&gt; tests</td>
<td></td>
</tr>
<tr>
<td>IVD</td>
<td>In Vitro Diagnostic Medical Device</td>
</tr>
<tr>
<td>Consult instruction for Use</td>
<td></td>
</tr>
<tr>
<td>CE mark</td>
<td></td>
</tr>
<tr>
<td>Keep away from sunlight and heat</td>
<td></td>
</tr>
</tbody>
</table>

BEP®, BEP 2000 Advance® and Enzygnost® are trademarks of Siemens Healthcare Diagnostics.

© 2009 Siemens Healthcare Diagnostics Products GmbH.

All rights reserved.

2015-04
### Table 1  Storage and Stability

<table>
<thead>
<tr>
<th>Material/reagent</th>
<th>State</th>
<th>Storage</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBs II MTP test plate, remaining strips</td>
<td>once opened</td>
<td>2–8 °C in the bag with desiccant</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Anti-HBs II Conj</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Anti-HBs II REF P</td>
<td>once opened</td>
<td>2–8 °C ≤ −20 °C</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Anti-HBs II Neg</td>
<td>once opened</td>
<td>2–8 °C ≥ −20 °C</td>
<td>3 months</td>
</tr>
<tr>
<td>Chromogen TMB</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Buffer/Substrate TMB</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td>Chromogen Working Solution</td>
<td>diluted 1+10</td>
<td>2–8 °C</td>
<td>5 days</td>
</tr>
<tr>
<td></td>
<td></td>
<td>15–25 °C protected from light</td>
<td>8 hours</td>
</tr>
<tr>
<td></td>
<td></td>
<td>in closed container</td>
<td></td>
</tr>
<tr>
<td>Washing Solution POD</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
<tr>
<td></td>
<td>diluted 1+19</td>
<td>2–8 °C</td>
<td>1 week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18–25 °C</td>
<td>1 day</td>
</tr>
<tr>
<td>Stopping Solution POD</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>expiry date</td>
</tr>
</tbody>
</table>

* use each component by the expiry date at the latest

### Table 2  Sensitivity

The sensitivity studies, using different sample populations with Anti-HBs positive status, yielded the following data:

<table>
<thead>
<tr>
<th>Sample population</th>
<th>Number of samples</th>
<th>Enzygnost® Anti-HBs II reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-HBs positive</td>
<td>216</td>
<td>215</td>
</tr>
<tr>
<td>HBV-vaccinated</td>
<td>539</td>
<td>533</td>
</tr>
</tbody>
</table>

### Table 3  Precision

In these studies, the samples were tested on 5 days in 8-fold replicates. The calculation of intra- and interassay variation coefficients (CV) was performed by analysis of variance.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Mean absorbance value (A)</th>
<th>Intraassay CV (%)</th>
<th>Interassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>P1</td>
<td>1.25</td>
<td>4.0</td>
<td>3.9</td>
</tr>
<tr>
<td>P2</td>
<td>1.65</td>
<td>3.2</td>
<td>3.2</td>
</tr>
<tr>
<td>P3</td>
<td>0.59</td>
<td>7.7</td>
<td>8.3</td>
</tr>
<tr>
<td>P4</td>
<td>0.28</td>
<td>3.7</td>
<td>12.7</td>
</tr>
</tbody>
</table>
Table 4  Test Procedure

Preparation of the reagents

Dispense 25 µL Anti-HBs II Conj

4 x 100 µL Anti-HBs II Neg
1 x 100 µL Anti-HBs II REF P
100 µL of each sample
1 x 100 µL Anti-HBs II REF P

Incubation: 60 ±2 minutes (37 ±1 °C)

Wash 4 x

100 µL Chromogen Working Solution

Incubation (protected from light): 30 ±2 minutes (15 to 25°C)

100 µL Stopping Solution

(after max. 60 minutes) Measure: 450 nm vs. 650 nm

in case of partially filled plates, add water-filled strips to half fill the plates

BEP® 2000 fully automated test processing

BEP® III automated test processing

Test result