**Intended Use**

Enzyme immunoassay for the qualitative detection of specific antibodies to Hepatitis B (core)-Antigen in human serum or 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**

Antibodies to hepatitis B (core) antigen (anti-HBc) are the first antibodies to appear in an acute hepatitis B infection. They occur shortly after the antigens HBsAg and HBeAg\(^1\), and often persist for life\(^2\). Consequently, the determination of anti-HBc in the serum can be utilized for monitoring the course of a hepatitis B infection\(^3\). Furthermore, anti-HBc can serve as a marker for the differential diagnosis of hepatitis A, hepatitis B, and non-A/non-B hepatitis. When the anti-HBc assay is used for screening purposes, a positive finding will indicate past contact with the hepatitis B virus even in cases of sera negative for HBsAg and anti-HBs. Approximately 10 % of all infections can be detected only by the serological determination of anti-HBc\(^4\).

Prior to the administration of hepatitis B vaccine, the anti-HBc test yields information on the immune status of the person to be vaccinated\(^5\).

In epidemiological studies the antibody to HBc antigen is a valuable parameter as it can be detected over a longer period of time than the antibody to HBs antigen\(^2\).

**Principle of the Method**

Enzygnost® Anti-HBc monoclonal is a competitive one-step enzyme immunoassay. The anti-HBc contained in the sample and the Anti-HBc/POD Conjugate compete for binding to the HBcAg coated onto the wells of the microtitration plate. 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. Due to the competitive principle of the test, the color intensity is inversely proportional to the concentration of anti-HBc in the sample.

**Reagents**

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Materials provided</th>
<th>2 x 96</th>
<th>10 x 96</th>
<th>10 x 96 (Q)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBC MONO</td>
<td>Enzygnost® Anti-HBc monoclonal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MTP</td>
<td>Enzygnost® Anti-HBc monoclonal test plate</td>
<td>2 pcs.</td>
<td>10 pcs.</td>
<td>10 pcs.</td>
</tr>
<tr>
<td>CONJUGATE</td>
<td>Anti-HBc/POD Conjugate monoclonal</td>
<td>2 x 1.2 mL</td>
<td>10 x 1.2 mL</td>
<td>2 x 3.2 mL</td>
</tr>
<tr>
<td>REAGENT DILUENT</td>
<td>Conjugate Buffer (anti-HBc monoclonal)</td>
<td>4 x 12.5 mL</td>
<td>10 x 12.5 mL</td>
<td>2 x 75 mL</td>
</tr>
</tbody>
</table>
### Materials provided

<table>
<thead>
<tr>
<th>Symbols</th>
<th>Materials provided</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL −</td>
<td>Anti-HBc Control Serum, negative</td>
<td>2 x 0.7 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 x 0.7 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 x 0.7 mL</td>
</tr>
<tr>
<td>CONTROL +</td>
<td>Anti-HBc Control Serum, positive</td>
<td>2 x 0.5 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 x 0.5 mL</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 x 0.5 mL</td>
</tr>
<tr>
<td>WASH POD</td>
<td>Washing Solution POD*</td>
<td>1 x 100 mL</td>
</tr>
<tr>
<td>SUBSTRATE TMB</td>
<td>Buffer/Substrate TMB*</td>
<td>1 x 30 mL</td>
</tr>
<tr>
<td>CHROMOGEN TMB</td>
<td>Chromogen TMB*</td>
<td>1 x 3 mL</td>
</tr>
<tr>
<td>STOP POD</td>
<td>Stopping Solution POD*</td>
<td>1 x 100 mL</td>
</tr>
<tr>
<td>EMPTY VIAL</td>
<td>Empty bottle for Chromogen Working Solution</td>
<td>1 pc.</td>
</tr>
<tr>
<td>CHROM SOL</td>
<td>Label &quot;EMPTY VIAL Enzygnost®&quot;</td>
<td>1 pc.</td>
</tr>
</tbody>
</table>

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

The test plate, the conjugate, the conjugate buffer, as well as Anti-HBc Control Serum, negative and Anti-HBc Control Serum, positive 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, if included in the package.

### Materials required but not provided, for the kits 10 x 96 and 10 x 96 (Q)

Supplementary Reagents for Enzygnost®/TMB (REF OUVP)

The reagents Chromogen TMB and Buffer/Substrate TMB must be used only in the combination of lots stated for the Supplementary Reagents kit. The applicable lot numbers are the 6-digit lot numbers listed on the package.

### Composition

**Enzygnost® Anti-HBc monoclonal test plate:** microtitration plate coated with recombinant hepatitis B core antigen

**Anti-HBc/POD Conjugate monoclonal:** monoclonal anti-HBc, peroxidase (POD)-conjugated

Preservative: phenol (≤ 1 g/L)

**Conjugate Buffer (anti-HBc monoclonal):** TRIS/HCl buffer with bovine serum and Tween 20

Preservative: phenol (≤ 1 g/L)

**Anti-HBc Control Serum, negative:** human serum without HBc-specific antibodies, stabilized

Preservatives: amphotericin (~ 5 mg/L)

gentamicin (~ 100 mg/L)

**Anti-HBc Control Serum, positive:** human serum with antibodies to HBc, stabilized

Preservatives: amphotericin (~ 5 mg/L)

gentamicin (~ 100 mg/L)

**Washing Solution POD:** 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:** sulphuric acid (0.25 mol/L)
Warnings and Precautions

For in-vitro diagnostic use.

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

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 liquids should 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 18 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.

To avoid a frequent change of syringes when processing large series of samples on the BEP® III System, the kit 10 x 96 (Q) ([REF OUWE 29](#)) is recommended.

When using kit variant 10 x 96 ([REF OUWE 17](#)), several vials of the conjugate can be transferred into a larger bottle (e.g. fresh, surplus empty bottle supplied for the Chromogen Working Solution from the kit Supplementary Reagents ([REF OUVP 17](#)), which is placed in the reagent rotor with the provided label "EMPTY VIAL..." for Anti-HBc/POD Conjugate monoclonal. Dilute Anti-HBc/POD Conjugate monoclonal 1+25 with Conjugate Buffer (anti-HBc monoclonal), e.g. for one test plate, add 0.5 mL conjugate into a vial with 12.5 mL Conjugate Buffer.
Buffer (anti-HBc monoclonal). Shake gently to mix. Accordingly, when using the kit
10 x 96 (Q) 3 mL of Anti-HBc/POD Conjugate monoclonal are added to a bottle with 75 mL
Conjugate Buffer (anti-HBc monoclonal) for 5 to 7 test plates depending on the dead volume
of the analyzer used. Document this step by using the check box on the label. Mix thoroughly,
e.g., by repeatedly inverting the bottle.

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
using the supplied empty plastic bottle (Chromogen Working Solution). Store protected from
light. After use, carefully rinse the bottle with distilled or deionized water. It is also permissible
to pour together the full contents of the Chromogen TMB vial and the Buffer/Substrate TMB
vial into the empty bottle.

When using Supplementary Reagents for Enzygnost®/TMB, [REF OUVP 29], the complete
contents of the Chromogen TMB vial have to be transferred into the barcode labeled Buffer/
Substrate TMB vial.

**Storage and Stability**

Stored unopened at the stated temperature, all components of the Enzygnost® Anti-HBc
monoclonal 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.

**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 multichannel 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)

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

**Specimens**

Suitable specimens are individual samples (human sera or citrated/EDTA/heparinized 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 frozen within this period, they can be stored at below −20 °C for at
least 1 year and 7 months if repeated freeze-thaw cycles are avoided.

**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 determination (n = 6) for Anti-HBc Control Serum, negative
and positive.

2. **Dispense samples:** Dispense 25 µL Anti-HBc Control Serum, negative, into each of 4 wells
(A1-D1), 25 µL Anti-HBc Control Serum, positive into one well (E1) and 25 µL of undiluted
sample into each of the subsequent wells. At the end of the series respectively test plate fill one further well with 25 µL Anti-HBc Control Serum, positive.

As an alternative to the pipetting scheme above, it is also possible to pipette Anti-HBc Control Serum, positive in duplicate after pipetting Anti-HBc Control Serum, negative.

**Alternative pipetting scheme:** Dispense 25 µL Anti-HBc Control Serum, negative into each of 4 wells (A1-D1), 25 µL Anti-HBc Control Serum, positive into 2 wells (E1-F1), and 25 µL of undiluted sample into each of the subsequent wells.

3. **Dispense conjugate:** Within 15 minutes after completing the sample dispensing step, pipette 100 µL of diluted Anti-HBc/POD Conjugate monoclonal into each well. Then seal the test plate with fresh foil and place immediately into the incubator.

4. **Incubate:** Incubate for 60 ±5 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 Chromogen Working Solution into each well, then seal the microtitration plate with fresh foil.

7. **Incubate substrate:** Immediately after the substrate dispensing step, incubate at 18 to 25 °C for 30 ±2 minutes, protected from light.

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

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 and 2 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 "Nonautomated 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 of the test 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-HBc Control Serum, negative: 0.700 ≤ A ≤ 2.500
2. Anti-HBc Control Serum, positive: -0.010 ≤ A ≤ 0.100

If one of the absorbance values of the Anti-HBc Control Serum, negative, is outside the specification, this value can be neglected.

Both absorbance values of the Anti-HBc Control Serum, positive must comply with 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.

Results

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

To calculate the cut-off, use the mean of the absorbance values of Anti-HBc Control Serum, negative and multiply with 0.2:

\[ \overline{A}_{\text{neg.}} \times 0.2 = \text{cut-off} \]

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

1. Anti-HBc negative \( A_{\text{sample}} > \text{cut-off} + 10 \% \)
2. Anti-HBc positive \( A_{\text{sample}} \leq \text{cut-off} \)
3. Anti-HBc equivocal cut-off < \( A_{\text{sample}} \leq \text{cut-off} + 10 \% \)

If an equivocal result is obtained, the sample is to be retested in duplicate. If, in the retest, both absorbance values are above or below the equivocal range, the initial equivocal result can be ignored and the sample can be considered negative or positive, respectively. However, if the sample reacts equivocally in one or both of the determinations of the retest, it is recommended to test a new sample collected 2 to 4 weeks after the first sample.

Limitations of the Procedure

1. Samples containing sodium azide must not be used!
2. Anticoagulants (heparin, EDTA, citrate) do not interfere with the test result.
3. No interferences have been observed with heat-treated samples (60 minutes, 56 °C).
4. Incompletely coagulated sera and microbially contaminated test samples should not be used. Any particles (e.g. fibrin clots, erythrocytes) contained in the sample should be removed prior to assay.
5. Samples that are haemolytic or contain rheumatoid factors do not impair the test results.
6. Samples containing antibodies to CMV, and anti-HBs positive samples, do not interfere with the test result.
7. Samples from patients with circulating immune complexes as well as samples containing anti-mouse IgG were not observed to interfere with the test result.
8. Samples containing antibodies to HAV, EBV, HIV, HCV as well as lipemic and icteric samples may exhibit elevated reactivity.
9. Samples from hemodialysis patients, transplant patients, patients with multiple blood transfusions as well as from patients with raised transaminase values may exhibit elevated reactivity.
10. If thawed samples are used, ensure that the material is thoroughly homogenized.
11. 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.
12. Highly reactive samples may cause a precipitation of the dye during the stopping reaction. This does not interfere with the photometric evaluation.
13. The control sera were produced using native human sera. Therefore, turbidity may occur but does not impair the test result.
14. 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 or use of the reagents on analyzers other than those included in Siemens Application Sheets or these Instructions for Use.

15. 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 results for the sensitivity and specificity study are summarized in Tables 2 and 3. The sensitivity of the test was studied using a total of 413 anti-HBc positive samples and the result obtained was 99.0 %. It cannot be ruled out that when the test is used on a large scale some samples may escape detection.

Analytical sensitivity was determined using a series of dilution of the WHO reference preparation as < 1.2 IU/L at the cut-off value.

The specificity of the test was studied using a total of 31,813 anti-HBc negative blood donation samples and the specificity results obtained were 99.6 % (initial testing) and 99.7 % (retest result).

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).

Current knowledge indicates that a positive result in the anti-HBc test is not a certain sign of HBV infection, just as a negative test result does not reliably exclude HBV infection.

Precision

Studies on the precision of Enzygnost® Anti-HBc monoclonal revealed intraassay coefficients of variation of 3.9 to 13.6 % and interassay coefficients of variation of 5.6 to 15.3 %. The calculation was performed using analysis of variance. For details refer to table 4.

Note

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

Bibliography

Definition of Symbols

- Do not reuse
- Use By
- Batch Code
- Catalogue Number
- Caution, consult accompanying documents
- Manufacturer
- Authorized representative in the European Community
- Contains sufficient for <n> tests
- In Vitro Diagnostic Medical Device
- Consult instruction for Use
- Non-sterile
- CE mark
- Reconstitution volume
- Keep away from sunlight and heat

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Table 1  Stability and Storage

<table>
<thead>
<tr>
<th>Material/reagent</th>
<th>State</th>
<th>Storage</th>
<th>Stability*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Enzygnost® Anti-HBc monoclonal 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-HBc/POD Conjugate monoclonal</td>
<td>once opened</td>
<td>2–8 °C ≤ −20 °C 2–8 °C 18–25 °C</td>
<td>4 weeks 3 months 4 weeks 1 week</td>
</tr>
<tr>
<td>Conjugate Buffer</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Anti-HBc Control Serum, positive</td>
<td>once opened</td>
<td>2–8 °C</td>
<td>4 weeks</td>
</tr>
<tr>
<td>Anti-HBc Control Serum, negative</td>
<td></td>
<td>≤ −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 15–25 °C closed container, protected from light</td>
<td>5 days 8 hours</td>
</tr>
<tr>
<td>Washing Solution POD</td>
<td>once opened</td>
<td>2–8 °C 18–25 °C</td>
<td>expiry date</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 performed at two independent centers (E, M) yielded the following data:

<table>
<thead>
<tr>
<th>Sample panel</th>
<th>Number of samples</th>
<th>positive</th>
</tr>
</thead>
<tbody>
<tr>
<td>(E) Acute HBV infection</td>
<td>26</td>
<td>26</td>
</tr>
<tr>
<td>Chronic HBV infection</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Different stages of HBV infection</td>
<td>31</td>
<td>28</td>
</tr>
<tr>
<td>(M) Acute HBV infection</td>
<td>194</td>
<td>194</td>
</tr>
<tr>
<td>Different stages of HBV infection</td>
<td>52</td>
<td>51</td>
</tr>
<tr>
<td>Follow-up samples</td>
<td>10</td>
<td>10</td>
</tr>
</tbody>
</table>

Table 3  Specificity

The specificity studies performed at three independent centers (B, L, S) yielded the following data:

<table>
<thead>
<tr>
<th>Sample panel</th>
<th>Number of sample</th>
<th>Initially reactive</th>
<th>Retest reactive</th>
</tr>
</thead>
<tbody>
<tr>
<td>(B) negative sera</td>
<td>17,872</td>
<td>65</td>
<td>57</td>
</tr>
<tr>
<td>(L) negative sera</td>
<td>3,949</td>
<td>13</td>
<td>10</td>
</tr>
<tr>
<td>(S) negative sera</td>
<td>9,992</td>
<td>34</td>
<td>29</td>
</tr>
</tbody>
</table>
### Table 4  Precision

Studies performed at two independent centers (T, Tr) yielded the following intraassay coefficients of variation (CV):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeats</th>
<th>Ratio</th>
<th>Intraassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T)</td>
<td>1</td>
<td>20</td>
<td>3.06</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>20</td>
<td>2.02</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>20</td>
<td>1.38</td>
</tr>
<tr>
<td>(Tr)</td>
<td>1</td>
<td>15</td>
<td>3.12</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>15</td>
<td>2.16</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>15</td>
<td>1.90</td>
</tr>
</tbody>
</table>

Studies performed at three independent centers (T, J, Tr) yielded the following interassay coefficients of variation (CV):

<table>
<thead>
<tr>
<th>Sample</th>
<th>Repeats</th>
<th>Ratio</th>
<th>Interassay CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>(T)</td>
<td>1</td>
<td>10</td>
<td>3.38</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>2.18</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10</td>
<td>1.69</td>
</tr>
<tr>
<td>(J)</td>
<td>1</td>
<td>10</td>
<td>3.76</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>10</td>
<td>2.66</td>
</tr>
<tr>
<td></td>
<td>3</td>
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<tr>
<td>(Tr)</td>
<td>1</td>
<td>7</td>
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<td></td>
<td>2</td>
<td>7</td>
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<td></td>
<td>3</td>
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<td>1.41</td>
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</tbody>
</table>

Ratio = absorbance / cut-off
Table 5  Test Procedure

Preparation of the reagents

4 x 25 µL Anti-HBc Control Serum, negative
2 x 25 µL Anti-HBc Control Serum, positive
25 µL of each undiluted sample

100 µL Conjugate Working Solution

Incubation:
60 ±5 minutes
(37 ±1 °C)

Wash 4 x

100 µL Chromogen Working Solution

Incubation (protected from light)
30 ±2 minutes
(18 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