Anti-HBc IgG ELISA

For the qualitative determination of anti-HBc total in human serum or plasma

Catalog Number: 68-4CBE3
Size: 96 wells

For Research Use Only. Not For Use In Diagnostic Procedures.

ALPCO Diagnostics
26G Keewaydin Drive • Salem, NH 03079
Phone: (800) 592-5726 • Fax: (603) 898-6854
www.alpco.com • Email: web@alpco.com
<table>
<thead>
<tr>
<th>Product Name</th>
<th>ANTICORASE B-96 (TMB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Product Code</td>
<td>4CBE3</td>
</tr>
<tr>
<td>Classification</td>
<td>Annex II, List A (98/79/EC)</td>
</tr>
<tr>
<td>Intended Use</td>
<td>For qualitative detection of total antibody to hepatitis B virus core antigen (anti-HBc total) in human serum or plasma</td>
</tr>
</tbody>
</table>

**Legal manufacturer**

GENERAL BIOLOGICALS CORPORATION

**Address**

#6, INNOVATION FIRST ROAD, SCIENCE- PARK, HSIN CHU, TAIWAN, R.O.C.

**E mail**

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886-3-5779221

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**Authorized Representative in EC**

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**Address**

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info@mt-procons.com
1) Intended Use

ANTICORASE B-96 (TMB) is an enzyme immunoassay for in vitro qualitative detection of total antibody to hepatitis B virus core antigen (Anti-HBc total) in human serum or plasma (heparin, EDTA or citrate).

2) Summary and Test Explanation

The hepatitis B virus (HBV) consists of an external envelope (HBsAg) and an inner core (HBeAg). In acute HBV infection, antibody to HBeAg (Anti-HBc total) is detectable in serum or plasma shortly before clinical symptoms and slightly after the appearance of HBsAg. In cases in which HBV infection resolves, anti-HBc appears in blood during the window period following loss of HBsAg and prior to the development of antibody to HBsAg (anti-HBs). Anti-HBc is found in acute and chronic hepatitis B patients and also indicates past resolved infection. Therefore, detection of anti-HBc is indicative of exposure to HBV and thus of infection by this virus. Further testing of HBV serological markers is required to define the specific disease state.1-6

ANTICORASE B-96 (TMB) is a fast test for the qualitative detection of the presence of antibodies to HBeAg in serum or plasma (heparin, citrate or EDTA) specimens. The test utilizes HBeAg on microtiter wells and human peroxidase-conjugated Anti-HBc in a competition principle to detect Anti-HBc levels in serum or plasma. Specimens with absorbance values greater than 1.1 x Cutoff Value are considered NEGATIVE for Anti-HBc. Specimens with absorbance values less than 0.9 x Cutoff Value are considered POSITIVE for Anti-HBc. The test has to be repeated in duplicate for specimens with absorbance value within the retest range (Cutoff Value ± 10 %) and interpreted as above. If the absorbance of any of the specimens retested in duplicate is still within the retest range, it is suggested to test follow-up samples of the patient.
3) Test Description

ANTICORASE B-96 (TMB) is a solid-phase enzyme immunoassay (ELISA) based on a competitive principle. The solid phase of the microtiter plate is made of polystyrene wells coated with HBcAg and the liquid phase of human peroxidase conjugated Anti-HBc.

When a serum or plasma specimen containing Anti-HBc is added to the HBcAg-coated wells together with the human peroxidase conjugated Anti-HBc and incubated, a competition will take place for the binding to the HBcAg on the wells. (HBcAg)-(Anti-HBc • peroxidase) complex and/or (HBcAg)-(Anti-HBc) complex will form on the wells. After washing the microtiter plate to remove unbound material, a solution of TMB substrate is added to the wells and incubated. Due to the competitive principle a color develops inversely proportional to the amount of Anti-HBc bound to HBcAg deriving from the specimen. The peroxidase-TMB reaction is stopped by addition of sulfuric acid. The optical density of developed color is read with a suitable photometer at 450 nm with a selected reference wavelength within 620 to 690 nm*8.

The above test principle is shown also in the following figure.

A Specimen containing Anti-HBc:
1. Plate well (HBcAg) + specimen (Anti-HBc) + (Anti-HBc • peroxidase) → Plate well (HBcAg • Anti-HBc) complex and (HBcAg • Anti-HBc • peroxidase) complex
2. Add TMB substrate solution → colorless ~ light blue
3. Add sulfuric acid to stop the color development → Read OD at 450 nm with a selected reference wavelength within 620 to 690 nm*8.

B Specimen without Anti-HBc:
1. Plate well (HBcAg) + specimen (without Anti-HBc) + (Anti-HBc • peroxidase) → Plate well (HBcAg • Anti-HBc • peroxidase) complex
2. Add TMB substrate solution → light blue ~ blue
3. Add sulfuric acid to stop the color development, read OD at 450 nm with a selected reference wavelength within 620 to 690 nm*8.
4) Description of Materials Provided & Product Code System

- Item 1 - 6 on the following reagent table should be refrigerated at +2 to +8°C. Conc. Washing Solution D (20X) and 2N H₂SO₄ can be stored at +2 to +30°C.

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>Material Code</th>
<th>Components</th>
<th>Description</th>
<th>Qt. per 96 tests</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>3B040MPCB</td>
<td>HBcAg Plate</td>
<td>Microtiter plate coated with HBcAg.</td>
<td>1 plate</td>
</tr>
<tr>
<td>(2)</td>
<td>3B071CONCBE3</td>
<td>Anti-HBc· Peroxidase Solution</td>
<td>Anti-HBc (human)· peroxidase conjugate dissolved in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>1 bottle 8 ml</td>
</tr>
<tr>
<td>(3)</td>
<td>3B090P3HCB3</td>
<td>Anti-HBc Positive Control</td>
<td>Anti-HBc positive serum in buffer with protein stabilizers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>1 bottle 1.5 ml</td>
</tr>
<tr>
<td>(4)</td>
<td>3B110N4HD3</td>
<td>HB Negative Control</td>
<td>Serum non-reactive for HBV markers. Preservatives: 0.003% Gentamycin and 0.01% Thimerosal.</td>
<td>1 bottle 2.0 ml</td>
</tr>
<tr>
<td>(5)</td>
<td>3B135TMB-A</td>
<td>TMB Substrate Solution A</td>
<td>0.6 mg/ml of 3,3',5,5'-tetramethylbenzidine (TMB) in an organic base.</td>
<td>1 bottle 12 ml</td>
</tr>
<tr>
<td>(6)</td>
<td>3B140TMB-B</td>
<td>TMB Substrate Solution B</td>
<td>Citrate acid buffer containing 0.03% H₂O₂.</td>
<td>1 bottle 12 ml</td>
</tr>
<tr>
<td>(7)</td>
<td>3B112PBS3</td>
<td>Conc. Washing Solution D (20X)</td>
<td>Concentrated phosphate buffer with Tween-20</td>
<td>1 bottle 58 ml</td>
</tr>
<tr>
<td>(8)</td>
<td>3B155SACID2N</td>
<td>2N Sulfuric Acid</td>
<td>2N H₂SO₄</td>
<td>1 bottle 12 ml</td>
</tr>
</tbody>
</table>

- ACCESSORIES: (provided as needed)

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>Material Code</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>(9)</td>
<td>2P951001</td>
<td>Adhesive Slips</td>
</tr>
<tr>
<td>(10)</td>
<td>2P505001</td>
<td>Absorbent Pads</td>
</tr>
<tr>
<td>(11)</td>
<td>2P403001</td>
<td>Black Cover</td>
</tr>
</tbody>
</table>

- OTHER MATERIAL REQUIRED, BUT NOT PROVIDED

<table>
<thead>
<tr>
<th>ITEMS</th>
<th>Components</th>
</tr>
</thead>
<tbody>
<tr>
<td>(1)</td>
<td>50 µl, 100 µl micropipettes and tips are needed</td>
</tr>
<tr>
<td>(2)</td>
<td>Incubator or waterbath with temperature control at +37 °C.</td>
</tr>
<tr>
<td>(3)</td>
<td>Plate washing equipment.</td>
</tr>
<tr>
<td>(4)</td>
<td>ELISA microwell reader: Dual wavelength 450nm with 620-690nm as reference wavelength*, bandwidth 10nm.</td>
</tr>
<tr>
<td>(5)</td>
<td>Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit.</td>
</tr>
</tbody>
</table>
4.1) Storage Conditions and Stability of Kit and Components

<table>
<thead>
<tr>
<th>Kit/Components</th>
<th>Storage condition</th>
<th>State</th>
<th>Stability</th>
</tr>
</thead>
<tbody>
<tr>
<td>ANTICORASE B-96 (TMB) KIT</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Anti-HBc Positive Control</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>HB Negative Control</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>2 months</td>
</tr>
<tr>
<td>HBcAg Plate</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Anti-HBc · Peroxidase Solution</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>18 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>Conc. Washing Solution D (20X)</td>
<td>Room temp.</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>20X Diluted Washing Solution</td>
<td>Room temp.</td>
<td>Diluted</td>
<td>2 days</td>
</tr>
<tr>
<td></td>
<td>+2~+8 °C</td>
<td>Diluted</td>
<td>1 week</td>
</tr>
<tr>
<td>TMB Substrate Solution A</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>TMB Substrate Solution B</td>
<td>+2~+8 °C</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
<tr>
<td>2N Sulfuric Acid</td>
<td>Room temp.</td>
<td>Original</td>
<td>24 months</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Once open</td>
<td>1 month</td>
</tr>
</tbody>
</table>

5) Instructions for Use

5.1) Warnings:

5.1.1) This reagent kit is for professional use only.

5.1.2) This reagent kit is for in vitro diagnostic use only.

5.1.3) Bring all kit reagents and samples to room temperature (+20 to +30°C) and mix carefully before use.

5.1.4) Do not use reagent beyond its expiration date.

5.1.5) Do not interchange reagents between different lots.

5.1.6) Do not pipette in the mouth.

5.1.7) Do not smoke or eat in areas where specimens or reagents are handled.

5.1.8) The positive control, negative control, conjugate solution and specimens should be regarded as potential hazards to health. They should be used and discarded according to the user’s laboratory safety procedures. Such safety procedures probably will include the wearing of protective gloves and avoiding the generation of aerosols.

5.1.9) Potential infectious specimens and nonacid containing spills or leakages should be wiped up thoroughly with 5% sodium hypochlorite or treated in accordance with the laboratory’s practice for potential bio-hazard control.

5.1.10) Prior to dispose the waste of used specimens and kit reagents as general waste, it should be treated in accordance with the local procedures for potential bio-hazardous waste or treated as follows: Both liquid and solid waste should be autoclaved maintaining +121°C for at least 30 minutes. Solid waste can also be incinerated. Non-acidic liquid waste can be treated with sodium hypochlorite diluted to a final concentration of 1%. Acidic liquid wastes must be neutralized before treatment with sodium hypochlorite as mentioned above and should stand for 30 minutes to obtain effective disinfection.
5.1.11) 2N sulfuric acid is an irritant to skin, eyes, respiratory tract and mucous membranes. Avoid contact of the 2N sulfuric acid with skin and mucous membranes. In case of contact, clean with large lots of water immediately. In case of inhalation, supply fresh air and seek medical advice in case of complaints.

5.1.12) TMB substrate solution A contains organic solvent, which is flammable. TMB substrate solution A contains dimethyl sulfoxide, an irritant to skin and mucous membranes.

5.1.13) Although all human sourced material are tested non-reactive for Anti-HCV and Anti-HIV, and inactivated at +56 °C for one hour, the reagent shall be handled as potential infectious material.

5.2) Specimen Collection and Preparation for Analysis

5.2.1) No special preparation of the patient is required prior to blood collection. Blood should be collected by approved medical techniques.

5.2.2) Either serum or plasma can be used with this diagnostic kit. Whole blood specimen should be separated as soon as possible in order to avoid hemolysis. Any particulates (e.g. fibrin clots, erythrocytes) contained in the specimen should be removed prior to use.

5.2.3) Specimens must be stored at +2 to +8°C and avoided heat-inactivation to minimize deterioration. For long-term storage, specimens should be frozen below -20°C. Storage in self-defrosting freezers is not recommended.

5.2.4) Frozen specimens must be thoroughly thawed and mixed homogenously before test.

5.2.5) Avoid multiple freeze-thaw procedures.

5.2.6) WARNINGS

1. The specimen must not contain any AZIDE compounds which can inhibit the peroxidase activity of the conjugate.

2. Incompletely coagulated serum samples and microbial-contaminated specimens should not be used.

5.3) Reagents Storage

5.3.1) The kit must be stored at +2 to +8°C. Do not freeze.

5.3.2) Strips of the plate should be used within 2 month after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped the opening tightly.

5.3.3) Return reagents to +2 to +8°C immediately after use.

5.3.4) Conc. Washing Solution D (20x) is stored and shipped at +2 to +8°C, which can cause crystallization. If the crystal has been precipitated before use, warm up the solution in +37°C water bath till the crystal is dissolved.

5.4) Plate Washing Procedure

5.4.1) Preparation of washing solution:
Dilute Conc. Washing Solution D (20X) with distilled or de-ionized water to 1:20 dilution. Do not use tap water.

5.4.2) Plate washing:
(a) For plate washer with overflow aspirating function: 6 cycles with at least 0.5ml washing buffer per well per cycle.

or

(b) For plate washer without overflow aspirating function: 8 cycles with at least 0.35ml washing buffer per well per cycle.

5.4.3) Blot dry by inverting the plate and tapping firmly onto absorbent paper. Too much residual wash buffer will cause false results.
WARNING
Improper washing will cause false results.

5.5) Test Procedure

5.5.1) Bring all reagents and specimens to room temperature (+20 to +30°C) before assay. Adjust water bath or incubator to +37±1°C.

5.5.2) Reserve 2 wells for blanks. Add 50 µl of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Positive Controls).

NOTE:
- Use a new pipette tip for each sampling to avoid cross-contamination.
- Each plate needs its own negative controls, positive controls and blank wells.
- Do not use cut-off value established for other plates of ANTICORASE B-96 (TMB).

5.5.3) Add 50 µl of Anti-HBc Peroxidase solution to each well except the 2 blanks.

NOTE:
- Do not touch the well wall for preventing contamination.

5.5.4) Gently tap the plate.

5.5.5) Remove the protective backing from the adhesive slip and press it onto the reaction plate, so that it is tightly sealed.

5.5.6) Incubate the reaction plate in a +37±1°C water bath or incubator for 1 hour.

5.5.7) At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with 5.4) Plate washing procedure.

5.5.8) Select one of the following two methods for color development:

A. Mix equal volumes of TMB Substrate Solution A and B in a clean container immediately prior to use. Add 100 µl of the mixture solution to each well including 2 blank wells.

B. Add 50 µl of TMB Substrate Solution A first, then add 50 µl of TMB Substrate Solution B into each well including the 2 blanks. Mix well gently.

NOTE:
- The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be protected from exposition to intense light.

5.5.9) Cover the plate with black cover and incubate at room temperature for 30 minutes.

5.5.10) Stop the reaction by adding 100 µl of 2N H2SO4 to each well including the two blanks.

5.5.11) Determine the absorbance of controls and test specimens within 30 minutes with a precision photometer at 450 / 620-690 nm (450 nm reading wavelength with 620-690 nm reference wavelength)\(^1\). Use the first blank well to blank the photometer.

NOTE: The blanks should be colorless to light yellowish in color; otherwise, the test results are invalid. In this case the tests must be repeated. **Substrate blank**: absorbance value must be less than 0.100.

5.6) Calculation of Test Results

5.6.1) Calculation of the NCx (Mean Absorbance of Negative Control).

Example:

<table>
<thead>
<tr>
<th>Sample No.</th>
<th>Absorbance</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.939</td>
</tr>
<tr>
<td>2</td>
<td>0.944</td>
</tr>
<tr>
<td>3</td>
<td>0.925</td>
</tr>
</tbody>
</table>

NCx = (0.939 + 0.944 + 0.925) / 3 = 0.936

**NCx must be ≥ 0.4, otherwise, the test run is invalid.**

5.6.2) Calculation of the PCx (Mean Absorbance of Positive Control)
Example:
Sample No. | Absorbance
--- | ---
1 | 0.068
2 | 0.052

\[ \text{PCx} = \frac{(0.068 + 0.052)}{2} = 0.060 \]

⚠️ PC \( x \) must be \( \leq 0.1 \), otherwise, the test run is invalid.

5.6.3) Calculation of the N - P Value
\[ \text{N - P} = \text{NCx} - \text{PCx} \]
Example:
\[ \text{N} - \text{P} = 0.936 - 0.060 = 0.876 \]

⚠️ N - P Value must be \( \geq 0.3 \), otherwise, the test run is invalid.

5.6.4) Calculation of the Cutoff Value
\[ \text{Cutoff Value} = 0.4 \text{ NCx} + 0.6 \text{ PCx} \]
Example:
\[ \text{Cutoff Value} = (0.4 \times 0.936) + (0.6 \times 0.060) = 0.410 \]

5.6.5) Calculation of the Retest Range
\[ \text{Retest Range} = \text{Cutoff Value} \pm 10\% \]
Example: Cutoff Value = 0.410
\[ \text{Retest Range} = (0.410 - 0.041) \text{ to } (0.410 + 0.041) = 0.369 \text{ to } 0.451 \]

5.7) ⚠️ Validity of Test Runs
5.7.1) \( \text{NCx} \) must be \( \geq 0.4 \), otherwise, the test run is invalid.
5.7.2) \( \text{PCx} \) must be \( \leq 0.1 \), otherwise, the test run is invalid.
5.7.3) \( \text{N-P Value} \) must be \( \geq 0.3 \), otherwise, the test is invalid.

5.8) Interpretation of Results
If the signal/cut-off ratio is within Retest Range (0.9-1.1 x cutoff), the test must be repeated in duplicate and interpreted as above. If both results are non-reactive the final result is non-reactive, if both results are reactive the final result is reactive. Any other combination is an indeterminate result. Testing of follow up samples and other hepatitis B serological markers should be taken into account in case of an indeterminate result.

5.9) Troubleshooting
If the result cannot be reproduced, preliminary troubleshooting should be performed by checking the possibilities listed below:
5.9.1) Improper washing procedure.
5.9.2) Contaminated with positive specimen.
5.9.3) Wrong volume of sample, conjugate or substrate.
5.9.4) Contamination of well rim with conjugate.
5.9.5) Improper specimen such as hemolyzed serum or plasma, specimen containing precipitate and specimen not thoroughly mixed before use.
5.9.6) Wrong incubation time or temperature.
5.9.7) Obstructed or partial obstructed washer aspirate/dispense head and needles.
5.9.8) Insufficient aspiration.

5.10) Limitations and Interferences
5.10.1) This reagent kit is to be used for un-pooled human serum or plasma samples only.
5.10.2) The reagent kit has not been validated for use with cadaveric samples.
5.10.3) Non-repeatable false positive results may be obtained with any enzyme immunoassay kit, largely due to technical error either on the part of the operator or malfunction of apparatus used.
5.10.4) Potential interfering substances:
Potential interfering samples, i.e. samples with hyperlipemia, hemolysis, hyper-bilirubinemia, and samples with monoclonal immunoglobulin components, samples containing elevated levels of autoimmune antibodies (rheumatoid factor-RF, antinuclear antibodies-ANA, or anti-mitochondrial antibodies-ANA) did not affect the test result with ANTICORASE B-96 (TMB).
5.10.5) The anticoagulants heparin, EDTA and sodium citrate have no influence on the specificity of ANTICORASE B-96 (TMB) and can be used to obtain plasma samples for analysis with the Anti-HBc Total kit.

5.11) Performance Characteristics

5.11.1) Diagnostic Specificity
Negative specimens/Specimens used to evaluate the specificity

<table>
<thead>
<tr>
<th>Type of sample</th>
<th>Number of samples</th>
<th>ANTICORASE B-96 (TMB)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood donor samples</td>
<td>5020</td>
<td>5010</td>
</tr>
<tr>
<td>Samples from hospitalized persons</td>
<td>200</td>
<td>200</td>
</tr>
<tr>
<td>Samples contain potential interfering factors</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>Samples with added possible interfering factors</td>
<td>12</td>
<td>11</td>
</tr>
<tr>
<td>Samples with different anticoagulants</td>
<td>48</td>
<td>48</td>
</tr>
<tr>
<td>Total</td>
<td>5377</td>
<td>5366</td>
</tr>
</tbody>
</table>

Diagnostic Specificity
5366/5377 = 99.8%

5.11.1.1) Potential interfering substances
Potential interferences with ANTICORASE B-96 (TMB) assay were investigated. For each potential interfering substance, at least two serum samples containing different amounts of the potentially interfering substance were mixed in fixed ratios of 10:0; 7:3; 5:5; 3:7; 0:10 with other serum samples containing increased Anti-HBc Total levels but no interfering factors. The neat samples as well as the mixtures were analyzed.

In particular the specificity study included:
- lipemic (turbid) samples (hyperlipidemia) before and after high speed centrifugation
- hemolytic samples or hemolysate
- icteric samples (=hyperbilirubinemia)
- samples with monoclonal immunoglobulin components (hyperimmunoglobulinemia)
- samples containing elevated levels of autoimmune antibodies (rheumatoid factor - RF, antinuclear antibodies –ANA, or antimitochondrial antibodies -AMA).

No interferences were detected with both used lots. Neither the type of anticoagulant had an influence on both tested lots of ANTICORASE B-96 (TMB).

5.11.2) Analytical Sensitivity and Linearity:
To evaluate the sensitivity of ANTICORASE B-96 (TMB) serial dilutions of the Standard Material for Anti-HBc Total of Paul Ehrlich Institute (PEI) (Langen, Germany) (100 PEI U/ml) were used.
For Lot# B34330PT: Linearity, R = -0.994
For Lot# B34331PT: Linearity, R = -0.991
Worst Case: Linearity, R = -0.991
Lot# A B
B34330PT 2.1397 -2.0009
B34331PT 2.0757 -1.8801
Lot# X=(Y-A)B
B34330PT Detection Limit = 1.858 PEI U/ml
B34331PT Detection Limit = 1.869 PEI U/ml
Worst Case Detection Limit = 1.869 PEI U/ml

The analytical sensitivity (detection limit) was defined as the lowest concentration which can be detected, i.e. at CO/S≥1.1 (i.e. S/CO≤0.9) calculated by using the linear regression function.

### 5.11.3) Diagnostic Sensitivity

#### 5.11.3.1) HBV infected individuals

435 HBV-positive samples were measured with both ANTICORASE B-96 (TMB) and the reference assay. The diagnostic sensitivity for the GBC assay was 100% as it was for the reference assay.

#### 5.11.3.2) Commercial seroconversion panels

Eight commercially available seroconversion panels consisting of follow-up samples which were collected at weekly or monthly intervals from patients suffering from acute hepatitis B, have been used. The panels were obtained from Boston Biomedica Inc., BBI; West Bridgewater, MA USA (PHM 933, PHM 934 and PHM 935A); Pyramid-Profile Diagnostics, Sherman Oaks, CA, USA (RP 009, RP 016 and RP 017) and NABI, Boca Roton, FL, USA (SB 411 and SB 413). All the panels have been characterized for HBV-specific serological markers (anti-HBs, anti-HBc IgM, anti-HBc-Total, and HBsAg).

By testing of the seroconversion panels GBC ANTICORASE B-96 (TMB) detected Anti-HBc Total one bleed earlier in the NABI panel RP-009 and the reference assay detected the Anti-HBc Total two bleeds earlier in the BBI Panel 935A and one bleed earlier in the NABI panel RP-017. In the other 5 panels GBC ANTICORASE B-96 (TMB) and the reference assay detected Anti-HBc Total in the same bleed.

In summary there was no significant difference between the GBC ANTICORASE B-96 (TMB) assay and the reference assay.

### 5.11.4) Precision

#### 5.11.4.1) Intra-run repeatability

For determination of intra-assay (within-run) precision, the Positive Control provided with the test kit and two patient serum samples with different Anti-HBc Total titer (slightly above the cutoff level and at medium level) were analyzed in replicates of 20 in a single “run” over 3 days. The CVs were in an acceptable range for both tested lots.

<table>
<thead>
<tr>
<th>Item tested</th>
<th>Sample size</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>N = 20</td>
<td>CV ≤ 12.68%</td>
</tr>
<tr>
<td>Patient Serum #1</td>
<td>N = 20</td>
<td>CV ≤ 10.62%</td>
</tr>
<tr>
<td>Patient Serum #2</td>
<td>N = 20</td>
<td>CV ≤ 16.72%</td>
</tr>
</tbody>
</table>

#### 5.11.4.2) Inter-run reproducibility

<table>
<thead>
<tr>
<th>Item tested</th>
<th>Sample size</th>
<th>Precision</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive Control</td>
<td>N = 60</td>
<td>CV ≤ 7.44%</td>
</tr>
<tr>
<td>Patient Serum #1</td>
<td>N = 60</td>
<td>CV ≤ 38.31%</td>
</tr>
<tr>
<td>Patient Serum #2</td>
<td>N = 60</td>
<td>CV ≤ 14.67%</td>
</tr>
</tbody>
</table>

### 5.11.5) Traceability

Concentration of Positive Control of ANTICORASE B-96 (TMB) referred to the PEI Anti-HBc Total Reference Material = 70 PEI U/ml ± 30%

### 5.11.6) Antibody Excess/High-Dose Hook Effect

The effect of antibody excess was tested by consecutive dilution of a standard material having very high Anti-HBc levels (PEI Anti-HBc Total Reference Material).
5.12) Flow Chart of Test Procedure

Add 50 µl controls (3 x NC, 2 x PC) and add 50 µl of each specimen into wells. Reserve 2 wells for blanks.

Add 50 µl of Anti-HBc·Peroxidase Solution into each reaction well, except 2 blanks.

Incubate the plate at +37±1°C for 1 hour.

Wash the plate.

(Choose one of the following two methods for color development)

Mix equal volumes of TMB Substrate Solution A and B. Add 100 µl of the mixed solution to wells.

Add 50 µl of TMB Substrate Solution A to wells and then add 50 µl of TMB Substrate Solution B. Mix well gently.

Incubate at R.T. for 30 minutes.

Add 100 µl of 2N H₂SO₄ into each well.

Determine absorbance using 450 nm as reading wavelength with 620-690 nm reference wavelength.

The Semilog PEI Standard Dilution chart illustrates that an antigen/antibody excess is not occurring also because of the reverse reaction used in this assay format. An antigen/antibody excess will not influence the reactive/non-reactive interpretation.
6) References

8. The reference wavelength of spectrometer can be 620nm to 690nm. However, user should validate the photometer in combination with this kit before use.