bi-x-act® of FINLAND

# HBE/Anti-Hbe ELISA 96 well plate Kit (TMB)

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Version 2532008

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## Intended Use

HBE/Anti-Hbe (TMB) is an enzyme immunoassay diagnostic kit, for in vitro qualitative testing HBeAg and Anti-HBe in human serum or plasma.

**Summary and Test Explanation**

**2.1) For HBeAg detection**

For HBeAg detection, HBE/ANTI-HBE (TMB) adopts the "sandwich principle" (Antibody·Antigen·Antibody) as the basis of the assay. When Anti-HBe coated wells and Anti-HBe·HRPO conjugate are incubated with specimens containing HBeAg, (antibody)-(antigen)-(antibody·HRPO) complexes are formed on the wells. After washing to remove unbound materials, TMB substrate is added and color develops in proportion to the amount of HBeAg bound. The color development is stopped by adding 2N sulfuric acid. The Optical Density of developed color is read with a suitable spectrophotometer against 450 nm/ 620- 690 nm*.

The above description is shown in the following figure.

**A Specimen containing HBeAg:**

(Plate well)-(Anti-HBe) + Specimen (HBeAg) + Anti-HBe·HRPO
→ (plate well)-Anti-HBe·HBeAg·(Anti-HBe·HRPO) sandwich complex
Sandwich complex + TMB substrate solution → Light blue to blue color
Add 2N H2SO4 to stop the color development →Read OD at 450 nm/ 620-690 nm*

**B Specimen without HBeAg:**

1. (Plate well)-(Anti-HBe) + Specimen (no HBeAg) + Anti-HBe·HRPO → (plate well)-Anti-HBe
(plate well)-Anti-HBe + TMB substrate solution → Colorless to light blue color
Add 2N H2SO4 to stop the color development →Read OD at 450 nm/ 620 -690 nm*

**2.2) For Anti-HBe detection**

For Anti-HBe detection, HBE/ANTI-HBE (TMB) adopts the "Neutralization Principle".

When specimen is incubated with Neutralizing Solution for Anti-HBe in the well(s) coated with Anti-HBe, The HBeAg from the the Neutralizing Solution for Anti-HBe will be neutralized by Anti-HBe in the specimen. The more the concentration of Anti-HBe, the less the concentration of remaining HBeAg, and finally the lower the Optical Density developed. The above description is shown in the following figure.

**A Specimen containing Anti-HBe:**

(plate well)-Anti-HBe + Specimen (Anti-HBe) + Neutralizing Solution (HBeAg)
→ (Plate well)-Anti-HBe·HBeAg and HBeAg·Anti-HBe
After washing, only (Plate well)-Anti-HBe·HBeAg remains on the plate well(s).
(Plate well)-Anti-HBe·HBeAg + Anti-HBe·HRPO
→ (Plate well)-Anti-HBe·HBeAg·(Anti-HBe·HRPO) sandwich complex
Sandwich complex + TMB substrate solution
→ Light blue to colorless
Add 2N H2SO4 to stop the color development

→ Read OD at 450 nm/ 620 - 690 nm*.

**B Specimen without Anti-HBe:**

Plate well (Anti-HBe) + Specimen (without Anti-HBe) + Neutralizing Solution (HBeAg)
→ (Plate well)-Anti-HBe·HBeAg
(Plate well)-Anti-HBe·HBeAg + Anti-HBe·HRPO → (Plate well) - Anti-HBe·HBeAg· (Anti-HBe·HRPO) sandwich complex
Sandwich complex + TMB substrate solution → blue color
Add 2N H2SO4 to stop the color development →Read OD at 450 nm/ 620-690 nm*.

**Brief Description of the Product**

The solid phase of HBE/ANTI-HBE (TMB) is made of polystyrene wells coated with Anti-HBe; Anti-HBe is used to prepare the Anti-HBe·peroxidase (horseradish) conjugate in the liquid-phase. In HBeAg detection, specimens, which are non-reactive by HBE/ANTI-HBE (TMB) are considered negative for HBeAg. Specimens with absorbance values LESS than 0.9 X Cut-off Value are considered NON-REACTIVE for HBeAg by the criteria of HBE/ANTI-HBE (TMB). Specimen with absorbance values GREATER than 1.1 X Cutoff Value is considered REACTIVE for HBeAg. Specimen with absorbance values within the Retest Range (Cut off Value ± 10%), the test must be repeated in duplicate and interpreted as above.

In Anti-HBe detection, specimens, which are non-reactive by HBE/ANTI-HBE (TMB) are considered negative for Anti-HBe. Specimens with absorbance values greater than 1.1 X Cutoff Value are considered NON-REACTIVE for Anti-HBe by the criteria of HBE/ANTI-HBE (TMB). Specimen with absorbance values less than 0.9 X Cutoff Value is considered REACTIVE for Anti-HBe. Specimen with absorbance values within the Retest Range (Cut off Value ± 10%), the test must be repeated in duplicate and interpreted as above.

**Description of Materials Provided & Product Code system**

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

| ITEMS | Components | Descriptiuon | Qt. per 96 tests |
|---|---|---|---|
| (1) | Anti-HBe Plate | One microtiter plate coated with antibody to HBeAg（Anti-HBe）. | 1 plate |
| (2) | Anti-HBe·Peroxidase Solution | Containing anti-HBe • Peroxidase (horseradish) conjugate dissolved in protein stabilizers. Preservatives: 0.003 % Gentamycin and 0.01 % Thimerosal. | 1bottle, 10 ml |
| (3) | HBeAg Positive Control | Containing HBeAg positive serum diluted in buffer with protein stabilizers. Preservatives: 0.003 % Gentamycin and 0.01 % Thimerosal. | 1 bottle, 1.0 ml |

| | | | |
|---|---|---|---|
| (4) | Anti-HBe Positive Control | Containing Anti-HBe positive serum dissolved in buffer with protein stabilizers.<br>Preservatives:<br>0.003 % Gentamycin and 0.01 % Thimerosal. | 1 bottle, 1.0 ml |
| (5) | HB Negative Control | Containing normal human serum, which is free of HBeAg, Anti-HBe and HBsAg.<br>Preservatives:<br>0.003 % Gentamycin and 0.01 % Thimerosal. | 1 bottle, 1.5 ml |
| (6) | Neutralizing Solution for Anti-HBe | Containing HBeAg positive serum diluted in buffer with protein stabilizers.<br>Preservatives:<br>0.003 % Gentamycin and 0.01 % Thimerosal. | 1 bottle, 5 ml |
| (7) | TMB Substrate Solution A | 0.6 mg/ml of 3,3', 5,5'-tetramethylbenzidine (TMB) in an organic base. | 1 bottle, 10 ml |
| (8) | TMB Substrate Solution B | Citrate Acid Buffer containing 0.03 % H2O2. | 1 bottle, 10 ml |
| (9) | Conc. Washing Solution D (20X) | Concentrated Phosphate buffer with tween-20 | 1 bottle, 52 ml |
| (10) | 2N Sulfuric Acid | 2NH2SO4 | 1 bottle, 12 ml |

● ACCESSORIES: (provided as needed)

| ITEMS | Components |
|---|---|
| (10) | Adhesive Slips |
| (11) | Absorbent Pads |
| (12) | Black Cover |

● OTHER MATERIALS REQUIRED, BUT NOT PROVIDED

| ITEMS | Components |
|---|---|
| (1) | 50µl, 100µl micropipettes and tips are needed |
| (2) | Incubator with temperature control at 37℃. |
| (3) | Plate washing equipment. |
| (4) | ELISA micro-plate reader:<br>Dual wavelength 450nm with 620-690nm* as reference wavelength, bandwidth 10nm. |
| (5) | Fully automatic EIA micro-plate analyzer is optional. User should validate the automatic EIA micro-plate analyzer in combination with the kit. |

**4.1) Storage Condition and Stability of the kit ***

| Kit/components | Storage condition | State | Stability |
|---|---|---|---|
| HBE/ANTI-HBE (TMB) KIT | +2 to +8 °C | Original | 10 months |
| | | Once open | 1 month |
| HBeAg Positive Control | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| HBeAg Negative Control | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| Anti-HBe Positive Control | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| HB Negative Control | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| Anti-HBe Plate | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| Anti-HBe Peroxidase Solution | +2 to +8 °C | Original | 10 months |
| | | Once open | 1 month |
| Neutralizing Solution for Anti-HBe | +2 to +8 °C | Original | 15 months |
| | | Once open | 1 month |
| Concentrated Washing Solution D (20X) | +2 to +8 °C | Original | 24 months |
| | | Once open | 1 month |
| 20X Diluted Washing Solution | Room temp. | Diluted | 2 days |
| | +2 to +8 °C | Diluted | 1 week |
| TMB subtract Solution A | +2 to +8 °C | Original | 18 months |
| | | Once open | 1 month |
| TMB Subtract solution B | +2 to +8 °C | Original | 18 months |
| | | Once open | 1 month |
| 2N Sulfuric Acid | +2 to +8 °C | Original | 24 months |
| | | Once open | 1 month |

**Instruction for Use**

**5.1) Warning:**

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℃) 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 put the pipette in ybi-x-act 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 health hazards. It should be used and discarded according to our own laboratory's safety procedures. Such safety procedures may 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 local procedures for potential bio-hazard control.
5.1.10) Prior to disposing 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.

**5.2) 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 (EDTA, Citrate or Heparine) 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, they should be frozen below -20℃. Storage in self-defrosting freezer 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) WARNING
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 one month after opening the original aluminum foil bag. The unused strips should be kept in the aluminum foil bag and taped tightly.
5.3.3) Return reagents to +2 to +8 °C immediately after use.
5.3.4) Washing Solution D (20x) Concentrate can be stored at room temperature to avoid crys-tallization.

**5.4) Plate washing procedure**

5.4.1) Preparation of washing solution:

Dilute Washing Solution D (20 x) Concentrate with distilled or de-ionized water to 1:20 dilution.

Do not use tap water!

Plate washing:

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 0.35 ml 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 in the wells will cause false results.

WARNING

Improper washing will cause false results.

**5.5) Test procedure**

Assay process can be performed by automatic EIA micro-plate immuno-analyzer, please set up the program according to the following 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.

(A) HBeAg Detection

5.5.2a) Reserve 2 wells for blanks. Add 100µl of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 HBeAg Positive Controls).

NOTE:

a. Use a clean pipette tip for each sampling to avoid cross-contamination

B.Each plate needs its own negative controls, positive controls and blank wells.

c. Do not use cut-off value of other plate.

5.5.3a) Gently tap the plate.

5.5.4a) Remove the protective backing of the adhesive slip and press it on the plate, so that it is tightly sealed.

5.5.5a) Incubate the plate in a 37±1℃incubator or water bath for 1 hbi-x-act.

5.5.6a) At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with wash the plate as described under plate washing procedure section 5.4.

5.5.7a) Add 100µ1 of Anti-HBe • Peroxidase Solution into each reaction well except 2 blanks.

NOTTE: Do not touch the well wall for preventing contamination.

5.5.8a) Gently tap the plate.

5.5.9a) Remove the protective backing from the adhesive slip and press it onto the reaction plate, so that it is tightly sealed.

5.5.10a) Incubate the reaction plate in a 37±1℃ water bath or incubator for 1 hbi-x-act.

5.5.11a) 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.

5.5.12a) 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 the blank well.
or
B) Add 50 µl of TMB Substrate Solution A first, and then add 50 µl of TMB Substrate Solution B into each well including the blank. Carefully mix well.
NOTE:
TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.
5.5.13a) Cover the plate with a black cover and incubate at room temperature for 15 minutes.
5.5.14a) Stop the reaction by adding 100µl of 2N $H_2SO_4$ to each well including the blank.
5.5.15a) 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)*. Use the blank well to blank photometer.
NOTE:
The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid.

(B) Anti-HBe Detection
5.5.2b) Reserve 2 wells for blanks. Add 50µl of each control or specimen to appropriate wells of reaction plate (3 Negative Controls and 2 Anti-HBe Positive Controls).
NOTE:
Use a clean 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 of other plate.
5.5.3b) Add 50µ1 of Neutralizing Solution for Anti-HBe into each well except 2 blank wells.
NOTE: Use an individual tip for each sample to avoid cross-contamination.
5.5.4b) Gently tap the plate.
5.5.5b) Remove the protective backing of the adhesive slip and press it on the plate, so that it is tightly sealed.
5.5.6b) Incubate the plate in a 37±1℃incubator or water bath for 1 h.
5.5.7b) At the end of the incubation period, remove and discard the adhesive slip and wash the plate in accordance with wash the plate as described under plate washing procedure section 5.4.
5.5.8b) Add 100µ1 of Anti-HBe • Peroxidase Solution into each reaction well except 2 blanks.
NOTE: Do not touch the well wall for preventing contamination.
5.5.9b) Gently tap the plate.
5.5.10b) Remove the protective backing from the adhesive slip and press it onto the reaction plate, so that it is tightly sealed.

5.5.11b) Incubate the reaction plate in a 37±1℃ water bath or incubator for 1 hbi-x-act.
5.5.12b) 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.
5.5.13b) 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 the blank well.
or
B) Add 50 µl of TMB Substrate Solution A first, and then add 50 µl of TMB Substrate Solution B into each well including the blank. Carefully mix well.
NOTE:
TMB Substrate Solution A should be colorless to light blue; otherwise, it should be discarded. The mixture of TMB Substrate Solution A and B should be used within 30 minutes after mix. The mixture should be avoided from intense light.
5.5.14b) Cover the plate with a black cover and incubate at room temperature for 15 minutes.
5.5.15b) Stop the reaction by adding 100µl of 2N $H_2SO_4$ to each well including the blank.
5.5.16b) 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)*. Use the blank well to blank photometer.
NOTE:
The color of the blank should be colorless to light yellowish; otherwise, the test results are invalid.

**5.6) Calculation of the tested data**
(A) HBeAg Detection
5.6.1a) Calculation of the NCx (Mean Absorbance of Negative Control).

| Example: Sample No. | Absorbance |
|---|---|
| 1 | 0.025 |
| 2 | 0.028 |
| 3 | 0.022 |

NCx = (0.025+0.028+0.022)/3 = 0.025
NCx must be ≦0.1, otherwise, the test is invalid.
5.6.2a) Calculation of the PCx (Mean Absorbance of Positive Control)

| Example: Sample No. | Absorbance |
|---|---|
| 1 | 1.246 |
| 2 | 1.202 |

PCx = (1.246 + 1.202) /2 = 1.224
PCx must be ≧0.4; otherwise, the test is invalid.
5.6.3a) Calculation of the P - N Value
P - N = PCx – NCx
Example: P - N = 1.224 - 0.025 = 1.224

P - N Value must be ≧ 0.3, otherwise, the test is invalid.

5.6.4a) Calculation of the Cutoff Value

Cutoff Value = NCx + 0.06

Example: Cutoff Value = 0.025 +0.06 = 0.085

5.6.5a) Calculation of the Retest Range

Retest Range = Cutoff Value ± 10 %

Retest Range = (0.085 - 0.009) to (0.085 + 0.009) = 0.076 to 0.094

5.6.6a) Quality Control of the Test Run

5.6.6.1a) NCx should be ≦ 0.1, otherwise, the test is invalid.

5.6.6.2a) PCx should be ≧ 0.4, otherwise, the test is invalid.

5.6.6.3a) P - N Value must be ≧ 0.3, otherwise, the test is invalid.

(B) Anti-HBe Detection

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

Example:

| Sample No. | Absorbance |
|---|---|
| 1 | 0.888 |
| 2 | 0.915 |
| 3 | 0.909 |

NCx =(0.888+0.915+0.909)/3= 0.904

NCx must be ≧0.4, otherwise, the test is invalid.

5.6.2b) Calculation of the PCx (Mean Absorbance of Positive Control)

Example:

| Sample No. | Absorbance |
|---|---|
| 1 | 0.044 |
| 2 | 0.056 |

PCx = (0.044+0.056)/2 = 0.050

PCx must ≦ 0.1; otherwise, the test is invalid.

5.6.3b) Calculation of the N - P Value

N - P = NCx –PCx

Example: N - P = 0.904 - 0.050 = 0.854

N – P Value must be ≧ 0.3, otherwise, the test is invalid.

5.6.4b) Calculation of the Cutoff Value

Cutoff Value = (NCx + PCx) /2

Example: Cutoff Value = Cutoff Value = (0.904 + 0.050) / 2 = 0.477

5.6.5b) Calculation of the Retest Range

Retest Range = Cutoff Value ± 10 %

Retest Range =(0.477 - 0.048) to (0.477 + 0.048) = 0.429 to 0.525

5.6.6b) Quality Control of the Test Run

5.6.6.1b) NCx should be ≧0.4, otherwise, the test is invalid.

5.6.6.2b) PCx should be ≦0.1, otherwise, the test is invalid.

5.6.6.3b) N - P Value must be ≧ 0.3, otherwise, the test is invalid.

**5.7) Result interpretation**

(A) HBeAg Detection

5.7.1a) Specimens with absorbance values less than (0.9 X Cutoff Value) are considered NON-REACTIVE and are considered NEGATIVE for HBeAg.

5.7.2a) Specimens with absorbance value greater than (1.1 X Cutoff Value) are considered REACTIVE and are considered POSITIVE for HBeAg.

5.7.3a) Specimens with absorbance value within the Retest Range (Cutoff Value ± 10 %) shall be repeated in duplicate and interpreted as above. Specimens with any of the repeat results in the retest range are reported as "indeterminate". It is suggested to test follow-up samples for "indeterminate" results.

(B) Anti-HBe Detection

5.7.1b) Specimens with absorbance values greater than (1.1 X Cutoff Value) are considered NON-REACTIVE and are considered NEGATIVE for Anti-HBe.

5.7.2b) Specimens with absorbance value less than (0.9 X Cutoff Value) are considered REACTIVE and are considered POSITIVE for Anti-HBe.

5.7.3b) Specimens with absorbance value within the Retest Range (Cutoff Value ± 10 %) shall be repeated in duplicate and interpreted as above. Specimens with any of the repeat results in the retest range are reported as "indeterminate". It is suggested to test follow-up samples for "indeterminate" results.

**5.8) Troubleshooting**

If the result cannot be reproduced, perform a preliminary troubleshooting by checking the possibilities listed below:

5.8.1) Improper washing procedure.

5.8.2) Contamination with positive specimen.

5.8.3) Wrong volume of sample, conjugate or substrates.

5.8.4) Contamination of the well rim with conjugate.

5.8.5) Improper specimen such as hemolyzed serum or plasma, specimen containing sediments and specimen not thoroughly mixed before use.

5.8.6) Wrong incubation time or temperature.

5.8.7) Obstructed or partial obstructed washer aspirate/dispense head and needles.

5.8.8) Insufficient aspiration.

**5.9) Limitations and Interferences**

5.9.1) This reagent kit is to be used for un-pooled human serum or plasma only.

5.9.2) The reagent kit has not been validated for use with cadaveric samples.

5.9.3) Non-repeatable false positive results may be obtained with any enzyme immunoassay kit, largely due to technical error either from the part of the operator or malfunction of apparatus used.

5.9.4) Repeatable false reactive results (≦2 %) may occasionally be obtained.
5.9.5) An Anti-HBe or HBeAg negative result without other evidence does not preclude the possibility of previous infection with hepatitis B virus.
5.9.6) A (low) positive result in the HBE/ANTI-HBE (TMB) Anti-HBe is no proof of protection and such it should be not used to exclude an infection by hepatitis B virus.
5.9.7) Anti-HBe positive specimens may not always show linear serial dilution properties as in serial dilution of standard material.
5.9.8) Potential Interfering Substances:
The following results were obtained in respective experiments:
No interferences with different anticoagulants such as lithium heparin, K-EDTA, sodium citrate have been observed.
Heat-treated specimens (+60°C, 10 hbi-x-acts) exhibited diminished HBsAg titer.
No cross reactivity was detected using specimens deriving from patients a) with other infections by HAV, EBV, CMV, HSV I, HSV II, Rubella, Toxoplasmosa, HCV, HIV, Anti-HBe(+), aHBe(+).
Samples containing potential interfering substances [e.g serum, anticoagulants EDTA, Citrate, Heparin and the lipmic, hemolytic, icteric samples with high monoclonal and elevated levels of autoimmune antibodies do not interfere with the test result] and samples from pregnant women did not interfere with the HBE/ANTI-HBE (TMB) assay.

**5.10) Performance characteristics**
5.10.1) HBeAg Assay
5.10.1.1) Analytical Specificity
Spiking experiment with HBeAg material performed with paired non-reactive serum and plasma samples with the three anticoagulants to show equivalence in the test results between serum and different types of plasma in the HBE/ANTI-HBE (TMB) test. The lipemic, hemolytic, icteric samples and samples with high monoclonal and elevated levels of autoimmune antibodies do not interfere with the test result. Pregnancy is not influencing the test result HBeAg. No false positive and false negative results are observed with samples with these characteristics.

5.10.1.2) Analytical Sensitivity
Two serial dilutions of the PEI HBeAg standard with lot B55C18PT: The calculated analytical sensitivity was 0.532 and 0.544 PEI Units/mL.
Two serial dilutions of the PEI HBeAg standard with lot B55C19PT: The calculated analytical sensitivity was 0.656 and 0.624 PEI Units/mL.

For HBeAg the values determined were 0.532; 0.544; 0.656; 0.624:
The mean for HBeAg sensitivity would be 0.59 PEI units/mL ± 0.06

5.10.1.3) Antigen Excess/High-dose hook effect

To test the antigen excess/high-dose hook effect a serum, which was spiked with HBeAg to a concentration of 10,000 U/mL, and was tested with the two evaluated lots if BI-X-ACT HBE/ANTI-HBE . No high-dose hook effect was detected with both tested lots.

5.10.1.4) Diagnostic Specificity

Results from the European Performance Evaluation for BI-X-ACT HBE/ANTI-HBE (TMB) HBeAg - Reactivity of HBV Negative "Donor" and "Clinical" Specimens.

| HBeAg negative Sample | BI-X-ACT | Abbott |
|---|---|---|
| Hospitalized patients | 111 | 112 |
| Potential interfering samples | 98 | 100 |
| Total | 209 | 212 |

Diagnostic specificity =111 /112= 99.12 %

The specificity of the assay for the clinical population is 99.12%.

5.10.1.5) Diagnostic sensitivity

5.10.1.5.1) HBV infected individuals

1. The diagnostic sensitivity determined in the European performance evaluations yielded the following results:

| Sample | No. of sample | Reactive | Sensitivity |
|---|---|---|---|
| HBeAg positive sera | 200 | 197 | 98.5% |

Diagnostic sensitivity = 197/200 = 98.5%

5.10.1.5.2) Commercial seroconversion panels

Two commercially available HBV seroconversion panels Profile Diagnostics RP-016 and RP-009, consisting of follow-up samples which were collected at weekly or monthly intervals from patients suffering from acute hepatitis B, were used. All the panels have been characterized for HBV-specific serological markers (HBeAg, Anti-HBe, anti-HBs, anti-HBc, anti-HBc-IgM, and HBsAg).

| Panel-ID | HBE/ANTI-HBE (TMB) | Abbott AxSym | Difference |
|---|---|---|---|
| PD RP-016 | 2 pos bleeds (days 57 and 60) | 2 pos bleeds (days 57 and 60) | No difference |
| PD RP-009 | 7 pos bleeds, 1 bleed in the gray zone (days 13 (GZ), 29, 31,36, 53, 56, 69, 81) | 9 pos bleeds, (days 11, 13, 29, 31,36, 53, 56, 69, 81) | Abbott Axsym was reactive 2 bleeds earlier or 2 days earlier. |

Summary of the evaluation of all tested Seroconversion Panels:

Panel RP-016 shows identical results whether in panel RP-009 the Abbott Axsym detects the first positive bleed at day 11 and the HBE/ANTI-HBE HBeAg has a result in the grey zone on day 13. This difference is not important in the routine diagnostics, because HBeAg is only a follow up parameter in the hepatitis B diagnostic and not a screening parameter.

5.10.1.6) Evaluation of Precision

5.10.1.6.1) Accuracy: intra-run repeatability and inter-run reproducibility

The positive control of the HBE/ANTI-HBE assay 44.0 PEI U/ml and one serum sample with HBeAg level just above cutoff and at medium level. The results were used to calculate the intra-run repeatability and inter-run reproducibility as presented in the following tables.

| Test Item | | Sample size | Test Result | Acceptance Range |
|---|---|---|---|---|
| Positive Control | intra-run | N = 22 | 2.12 % | CV of COI ≦ 15 % |
| | | N = 22 | 2.42 % | |
| | | N = 22 | 3.15 % | |
| | inter-run | N = 66 | 2.76 % | CV of COI ≦ 25 % |
| PC/4 | intra-run | N = 36 | 4.90 % | CV of COI ≦ 25 % |
| | | N = 36 | 4.42 % | |
| | | N = 36 | 5.68 % | |
| | inter-run | N = 108 | 5.28 % | CV of COI ≦ 30 % |

5.10.1.7) Traceability

BI-X-ACT HBeAg Master Calibrator has been calibrated against the PEI HBeAg Standard using the HBE/ANTI-HBE (TMB) assay. The relative potency (ratio) of the Paul Ehrlich Institute (PEI) Standard for HBeAg versus the BI-X-ACT HBeAg Master Calibrator is 1.187 (1.102-1.281 95% CI). The concentration of the Positive Control of HBE/ANTI-HBE (TMB) assay has been determined against the BI-X-ACT HBeAg Master Calibrator and was established with 44.0 PEI U/ml.

5.10.2) Anti-HBe Assay

5.10.2.1) Analytical Specificity

Spiking experiment with Anti-HBe material performed with paired non-reactive serum and plasma samples with the three anticoagulants to show equivalence in the test results between serum and different types of plasma in the HBE/ANTI-HBE (TMB) test. The lipemic, hemolytic, icteric samples and samples with high monoclonal and elevated levels of autoimmune antibodies do not interfere with the test result. Pregnancy is not influencing the test result Anti-HBe. No false positive and false negative results are observed with samples with these characteristics.

5.10.2.2) Analytical Sensitivity

Two serial dilutions of the PEI Anti-HBe standard with lot B55C18PT: The calculated analytical sensitivity was 0.30 PEI Units/mL.

Two serial dilutions of the PEI Anti-HBe standard with lot B55C19PT: The calculated analytical sensitivity was 0.30 PEI Units/mL.

For Anti-HBe the values determined were 0.30; 0.30; 0.26; 0.34:

The mean for Anti-HBe sensitivity would be 0.3 PEI units/mL ± 0.033

5.10.2.3) Antigen Excess/High-dose hook effect

Due to the competitive assay format a high-does hook effect can not occur.

5.10.2.4) Diagnostic Specificity

Results from the European Performance Evaluation for BI-X-ACT HBE/ANTI-HBE (TMB) Anti-HBe - Reactivity of HBV Negative "Donor" and "Clinical" Specimens.

| Anti-HBe negative Sample | BI-X-ACT | Abbott |
|---|---|---|
| Unselected samples | 200 | 200 |
| Hospitalized patients | 196 | 197 |

Diagnostic specificity =196 /197= 99.49 %

The specificity of the assay for the clinical population is 99.49%.

5.10.2.5) Diagnostic sensitivity

5.10.2.5.1) HBV infected individuals

1. The diagnostic sensitivity determined in the European performance evaluations yielded the following results:

| Sample | No. of sample | Reactive | Sensitivity |
|---|---|---|---|
| HBeAg positive sera | 201 | 199 | 99.0% |

Diagnostic sensitivity = 199/201 = 99.0%

5.10.2.5.2) Commercial of the seroconversion panels:

Two commercially available HBV seroconversion panels Profile Diagnostics RP-016 and RP-009, consisting of follow-up samples which were collected at weekly or monthly intervals from patients suffering from acute hepatitis B, were used. All the panels have been characterized for HBV-specific serological markers (HBeAg, Anti-HBe, anti-HBs, anti-HBc, anti-HBc-IgM, and HBsAg).

| Panel-ID | HBE/ANTI-HBE (TMB) | Abbott AxSym | Difference |
|---|---|---|---|
| RP-016 | 8 pos bleeds (days 107, 109, 114, 116, 121, 123, 128, 157 ) | 10 pos bleeds (days 81, 88, 107, 109,114, 116, 121, 123, 128,157) | Abbott AxSym was reactive 2 bleeds earlier or 26 days earlier. |
| RP-009 | 7 pos bleeds (days 88, 98, 109, 123, 133, 166, 186) | 9 pos bleeds, (days 88, 98, 109, 123,133, 152, 166, 186, 202) | Abbott AxSym was reactive at to more bleeds at day 152 und 186 |

Summary of the evaluation of all tested Seroconversion Panels:

Panel RP-016 shows difference of two bleeds in detecting Anti-HBe seroconversion where in panel RP-009 the Abbott AxSym detected the first positive bleed and the HBE/ANTI-HBE Anti-HBe detected the seroconversion the same bleed, but is two more bleeds reactive. This difference is not important in the routine diagnostics, because Anti-HBe is only a follow up parameter in the hepatitis B diagnostic and not a screening parameter.

5.10.2.6) Evaluation of Precision

5.10.2.6.1) Accuracy: intra-run repeatability and inter-run reproducibility

The negative control of the HBE/ANTI-HBE assay and one serum sample with Anti-HBe level just above cutoff and at medium level. The results were used to calculate the intra-run repeatability and inter-run reproducibility as presented in the following tables.

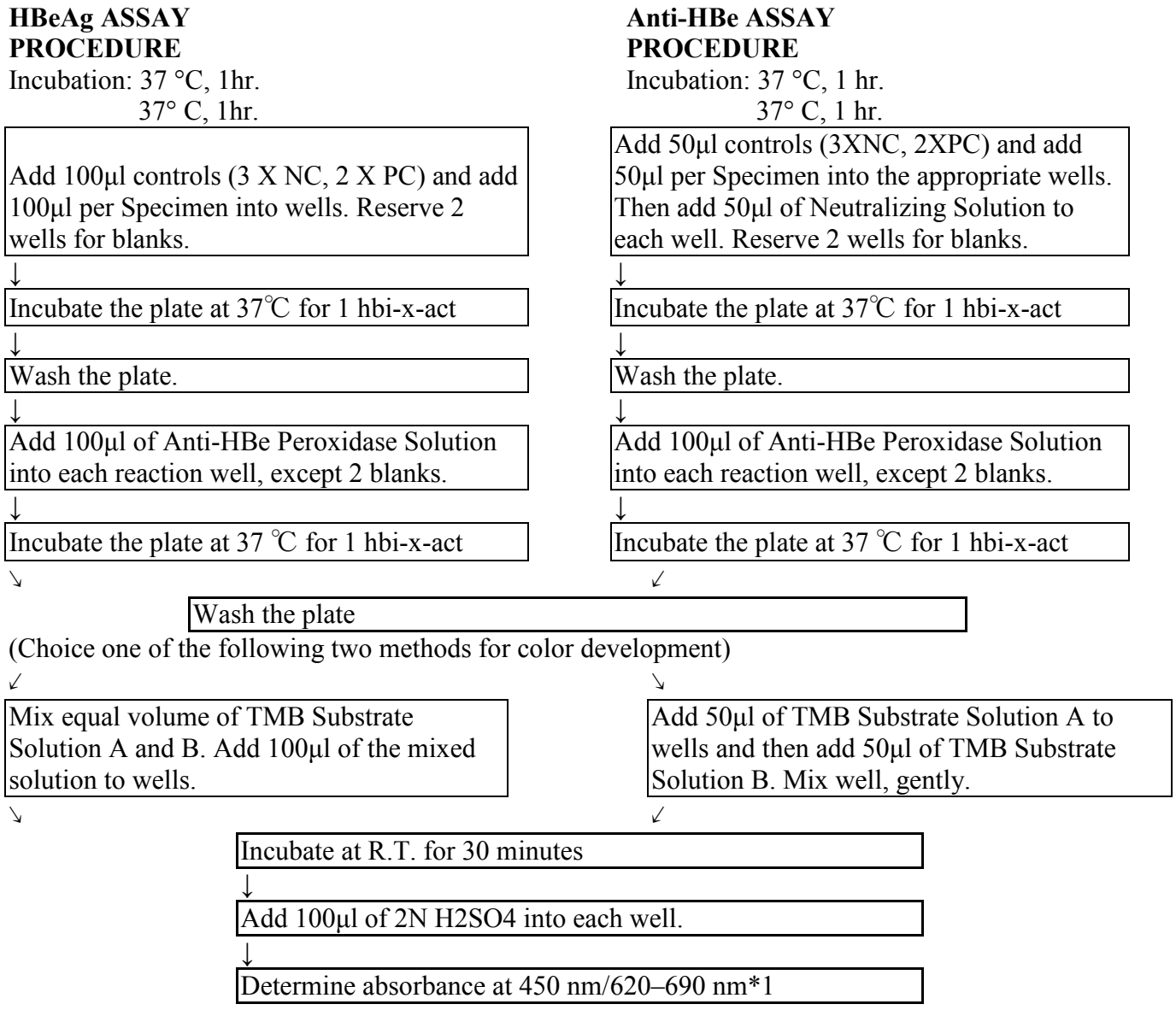
| Test Item | | Sample size | Test Result | Acceptance Range |
|---|---|---|---|---|
| Negative Control | intra-run | N = 36 | 3.71 % | CV of COI ≦ 15 % |
| | | N = 36 | 3.39 % | |
| | | N = 36 | 4.03 % | |
| | inter-run | N = 108 | 2.78 % | CV of COI ≦ 15 % |
| PC/20 | intra-run | N = 36 | 7.40 % | CV of COI ≦ 25 % |
| | | N = 36 | 7.51 % | |
| | | N = 36 | 7.15 % | |
| | inter-run | N = 108 | 5.51 % | CV of COI ≦ 25 % |

5.10.2.7) Traceability

BI-X-ACT Anti-HBe Master Calibrator has been calibrated against the PEI Anti-HBe Standard using the HBE/ANTI-HBE (TMB) assay. The relative potency (ratio) of the Paul Ehrlich Institute (PEI) Standard for Anti-HBe versus the BI-X-ACT Anti-HBe Master Calibrator is 1.115 (1.068-1.162 95% CI). The concentration of the Positive Control of HBE/ANTI-HBE (TMB) assay has been determined against the BI-X-ACT Anti-HBe Master Calibrator and was established with 9.0 PEI U/ml.

| 5.11) | Flow chart of the test procedure |
|---|---|

The Simplified procedure is only for the experienced users. New users are advised to read and follow the detailed. Test procedure carefully.




6) Bibliography

*1 The reference wavelength of the photometer to be used can be 620 nm to 690 nm. However, the user should validate the photometer in combination with HBE/ANTI-HBE (TMB) before use.

*2 Incomplete inactivation of hepatitis B virus after heat treatment at +60℃ for 10 hbi-x-acts, J. Infect. Dis. 138:242-244.