INTENDED USE

BeneSphera™ HBsAg is an in vitro enzyme immuno) assay for the qualitative detection of hepatitis B surface Antigen in Human Serum or Plasma.

INTRODUCTION

Hepatitis B virus (HBV) has been shown to be the viral agent responsible for Hepatitis B and has been linked to the development of primary hepatocellular carcinomas.

The presence of a component of the HBV, known as the hepatitis B surface antigen (HBsAg) in the serum indicates either a chronic or acute infection with virus.

A number of assays for HBsAg are available, many of them are using polyclonal Anti-HBs antibodies as a tool.

BeneSphera™ HBsAg kit represents a new generation of HBsAg assays in which specific monoclonal Anti-HBs antibodies are used. This assay promises to be more sensitive, convenient and safer to use, and has advantage of short reaction time as well as easier detection of target materials.

PRINCIPLE OF THE TEST

The principle of BeneSphera™ HBsAg is based on a direct, non-competitive, solid-phase enzyme immunoassay with horseradish peroxidase as the marker enzyme. The assay proceeds according to the following reactions:

1. HBsAg (●) when present in patient’s serum, combines with the mouse monoclonal anti-HBs (-○) antibodies coated on the polystyrene surface ( ), of the microstrip wells and simultaneously binds with the horse-radish peroxidase conjugated goat poly anti-HBs ( – △ ) antibodies.

2. After incubation, wells are washed and a colourless substrate (H₂O₂) chromogen (TMB) solution is added. The enzyme action on substrate/chromogen produces a coloured end product.

3. The enzyme-substrate/chromogen reaction is terminated with addition of 1.6 N H₂SO₄. The colour intensity is directly related to the concentration of hepatitis B surface antigen in the patient sample.

KIT CONTENTS:

1. Monoclonal Anti-HBs Coated Microstrips :
   1 well contains
   Mouse monoclonal Anti HBs ...................................................... q.s.
   Phosphate buffer (as solvent) ................................................................. q.s.

2a. Negative Control :
   1 bottle contains
   Normal human serum ................................................................. q.s.
   Kathon CG (as preservative) .............................................................. 0.1 %v/v

2b. Positive Control :
   1 bottle contains
   Hepatitis B Surface
   Inactivated Antigen (HBsAg)
   human serum ................................................................. q.s.
   Kathon CG (as preservative) .............................................................. 0.1 %v/v

3a. Conjugate Concentrate :50x
   1 bottle contains
   Anti HBsAg - Antibody
   peroxidase conjugate ........................................................................ q.s.
   Bovine serum (as protein stabilizer) ............................................ q.s.
   Kathon CG (as a preservative) ......................................................... 0.05 %v/v

3b. Conjugate Diluent:
   1 bottle contains
   Phosphate buffer .............................................................................. q.s.
   Goat serum (as protein stabilizer) ..................................................... q.s.
   Tween 20 (as surfactant) ................................................................. q.s.
   Kathon CG (as preservative) .............................................................. 0.05 %v/v

4a. TMB Chromogen in DMSO (101 x concentrated) :
   1 bottle contains
   Tetramethyl benzidine 3,3,5,5 (TMB) ........................................ q.s.
   Dimethyl sulfoxide, DMSO (as solvent) .................................................... q.s.

4b. Substrate Buffer:
   1 bottle contains
   30% Hydrogen peroxide .............................................................. 0.006 %v/v
   Citrate-acetate buffer ........................................................................ q.s.

5. Wash Solution (20 x concentrated) :
   1 bottle contains
   Concentrated phosphate buffer saline ........................................ q.s.
   Tween 20 (as surfactant) ................................................................. q.s.
   Kathon CG (as a preservative) .............................................................. 0.05 %v/v

6. Stop Solution:
   1 bottle contains
   1.6 N Sulfuric acid ........................................................................... q.s.

7. Empty zip seal polybag:
   (for storage of unused microstrips)

8. Plate Sealer in zip seal polybag : (Plastic sheets to cover microstrips during incubation)

9. Package Insert

MATERIALS REQUIRED BUT NOT PROVIDED

- Distilled or deionized water.
- Graduated cylinders for reagent dilutions.
- Micropipettes.
- Paper towels or absorbent paper. - Timer.
- Incubator/shaker, maintained at 37°C±1°C.
- ELISA Reader
- ELISA Washer.
- Sodium hypochlorite solution (free available chlorine 50-500 mg/l).
- Disposable gloves.

SPECIMEN COLLECTION, HANDLING AND STORAGE

- Serum and plasma (citrate or EDTA) samples may be stored up to 7 days at 2-8°C or at least 6 months as frozen (-20°C to -70°C). Samples should not be repeatedly frozen and thawed.
- Heat inactivated serum or plasma specimens may cause false HBsAg reactive results.
Do not use sodium azide as preservative because it inactivates horseradish peroxidase

- Microbially contaminated, grossly hemolyzed, icteric or hyperlipemic serum and plasma specimens may give erroneous results.

PRECAUTIONS

1) The material is for in vitro diagnostic use only [IVD].
2) Handle specimens carefully for capability of infection of unknown viruses.
3) Contamination of sample by microorganisms or heavy hemolysis can cause incorrect results.
4) Use rubber gloves in handling potentially biohazardous materials, and wash your hands with running water after handling.
5) Do not allow substrate to come in contact with skin.
6) Avoid microbial contamination of reagents when opening and removing aliquots from the vials.
7) Store at 2-8°C the remaining reagents.
8) Dispose off all specimens and materials used in testing by autoclaving for a minimum of 1 hour at 121°C.
9) Liquid/water which was used should be discarded after mixing it with 0.5% sodium hypochlorite for more than 1 hour.
10) Specimens should be stored at 2-8°C up to seven days and -20°C to -70°C for long term storage.
11) We recommend to use disposable plastic containers because soap or oxidising agent remaining in containers used for preparation of substrate TMB solution can interfere with the reaction. If reusable containers are used to prepare the solution they should be washed with 1 N H2SO4 or HCI. Rinsed well with de-ionised water and dried before use.

PREPARATION OF THE WORKING REAGENTS

1. Bring all the reagents to room temperature for 30 minutes before use.
2. Take the required number of strips from sealed monoclonal anti-HBs coated Plate. The remaining strips must be kept at 2-8°C with a silica gel in a vinyl wrapper, and it must be ensured that no moisture is permitted into the vinyl wrapper.
3. Preparation of Washing Solution
   Dilute washing solution 1:20 with distilled water or deionized water. For example, add 10ml of washing solution to 190 ml of deionized water.
   * Washing solution may be crystallized at cool storage condition.
   If crystallized, use it after thawing at 37°C.
4. Preparation of Conjugate
   Dilute conjugate 1:50 with conjugate diluent to the extent of required amount before 10 minutes of its use (Refer Table 1).
   * Conjugate diluent may be precipitated, therefore, shake well before use.

<table>
<thead>
<tr>
<th>Table 1.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strips required</td>
</tr>
<tr>
<td>Conjugate diluent (ml)</td>
</tr>
<tr>
<td>Concentrated conjugate (µl)</td>
</tr>
</tbody>
</table>

5. Preparation of substrate
   Dilute TMB Chromogen in DMSO 1:101 with substrate buffer for 5 to 10 minutes before use (Refer Table 2).
   * Avoid exposure to light.
   * Substrate should be used only after thawing at 37°C, if crystallized.

<table>
<thead>
<tr>
<th>Table 2.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strips required</td>
</tr>
<tr>
<td>Substrate Buffer (ml)</td>
</tr>
<tr>
<td>TMB Chromogen(µl)</td>
</tr>
</tbody>
</table>

STORAGE AND EXPIRY OF PREPARED REAGENT

<table>
<thead>
<tr>
<th>Prepared Reagent</th>
<th>Condition</th>
<th>Expiry</th>
</tr>
</thead>
<tbody>
<tr>
<td>Substrate Solution</td>
<td>RT (21-25°C)</td>
<td>0.5 hours</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>RT (21-25°C)</td>
<td>8 hours</td>
</tr>
<tr>
<td>Washing Solution</td>
<td>2-8°C</td>
<td>14 days</td>
</tr>
<tr>
<td>Conjugate</td>
<td>RT (21-25°C)</td>
<td>1 hours</td>
</tr>
</tbody>
</table>

TEST PROCEDURE

1. Fix the required numbers of strips to the frame.
2. Mix conjugate and conjugate diluent in the ratio of 1:50.
3. Pipette 50 µl of negative control into each well of 1A to 1C and 50 µl of positive control into well of 1D and 1E, respectively, and then pipette 50 µl of each specimen into the remaining wells.
4. Pipette 50 µl of prepared conjugate (of procedure 2) into each well and tap the frame gently to mix completely, and then incubate at 37 ± 1°C for 60 minutes.
5. Before the last 5 to 10 minutes (of the procedure 4) mix substrate solution and substrate buffer at the volume ratio of 1:101.
6. Aspirate the contents from all wells and wash each one 5 times with diluted washing solution (NLT 300 µl/well/time).
7. Invert the plate and tap it dry on absorbent paper. Pipette 100 µl of substrate solution (of procedure 5) into each well and incubate at controlled room temperature (21-25°C) for 30 minutes after mixing it with gentle tap.
8. Pipette 50 µl of stop solution into each well and shake well until the blue colour of each well turns into yellow colour.
9. Read the absorbance of positive, negative control and specimen at 450 nm (reference wavelength 620 nm) against air within 15 minutes.

QUALITY CONTROL

1) Negative control mean (NCx) should be less than or equal to 0.200.
2) Positive control mean should be greater than or equal to 1.00.
3) If the results are outside the above range, the test should be repeated.

INTERPRETATION OF RESULTS

(1) Calculation of the cut-off value
(a) Calculate the negative control mean (NCx)
   ex) negative control 1 absorbance 0.031
   negative control 2 absorbance 0.033
   negative control 3 absorbance 0.032
   *negative control mean (NCx) = (0.031 + 0.033 + 0.032)/3 = 0.032
(b) Calculate the cut-off value
Cut-off value = NCx + 0.100 = 0.032 + 0.1
= 0.132

(2) Interpretation
Samples with absorbance equal to or greater than the cut off value are considered positive to HBsAg. Samples with absorbance less than the cut off value are considered negative to HBsAg.

- If the samples are considered positive, the test should be conducted two more times. In case that the re-tests show negative, the samples are considered negative, and on the other hand, if one of the retests shows positive, the samples are considered positive.
- The samples considered positive should be tested again by confirmatory test for final judgement.

PERFORMANCE CHARACTERISTICS

1. Analytical sensitivity
Analytical sensitivity of BeneSphera™ HBsAg was 0.2 IU/ml.

2. Diagnostic sensitivity
Sensitivity of BeneSphera™ HBsAg was 100% (180/180).

3. Diagnostic specificity
Specificity of BeneSphera™ HBsAg was 99.5% (796/800).

4. Analytical specificity
BeneSphera™ HBsAg has no cross reactivity with Rheumatoid factor, Pregnancy, /Cold Antibody, ANA, EBV IGG, EBV, IgM, CMV Ig, CMV IgM, HEV IgG, HEV IgM, HAV-toal antibody, HAV IgM, Bilirubin (2.0-15.4 mg/dl), Haemoglobin (7-283mg/dl) and Triglyceride (246-1355 mg/dl) of interfering factors.

5. Assay reproducibility

1) Interassay, Intra assay
To test reproducibility of BeneSphera™ HBsAg when several chemist performed against the same specimen repeatedly. The results of the study are summarized in table.

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Number of Chemist</th>
<th>Average of Mean Absorbance</th>
<th>% CV</th>
<th>Number of assays</th>
<th>Average of Mean Absorbance</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>H274</td>
<td>5</td>
<td>0.686</td>
<td>8.11</td>
<td>8</td>
<td>0.692</td>
<td>6.63</td>
</tr>
<tr>
<td>H275</td>
<td>5</td>
<td>2.127</td>
<td>5.66</td>
<td>8</td>
<td>2.171</td>
<td>4.64</td>
</tr>
<tr>
<td>H276</td>
<td>5</td>
<td>1.882</td>
<td>5.60</td>
<td>8</td>
<td>1.872</td>
<td>4.13</td>
</tr>
<tr>
<td>H278</td>
<td>5</td>
<td>0.931</td>
<td>5.17</td>
<td>8</td>
<td>0.888</td>
<td>6.17</td>
</tr>
<tr>
<td>H280</td>
<td>5</td>
<td>0.414</td>
<td>6.62</td>
<td>8</td>
<td>0.333</td>
<td>7.97</td>
</tr>
<tr>
<td>H281</td>
<td>5</td>
<td>0.208</td>
<td>9.91</td>
<td>8</td>
<td>0.228</td>
<td>8.41</td>
</tr>
</tbody>
</table>

2) Reproducibility among batches
To test reproducibility among batches of plate, The plates of 3 production batches each were tested 3-times against a Quality control panel of HBsAg positive and negative samples. The results of the study are summarized in table.

<table>
<thead>
<tr>
<th>Specimen No.</th>
<th>Batch 1</th>
<th>Batch 2</th>
<th>Batch 3</th>
<th>Average</th>
<th>% CV</th>
</tr>
</thead>
<tbody>
<tr>
<td>H372</td>
<td>0.506</td>
<td>0.483</td>
<td>0.466</td>
<td>0.485</td>
<td>7.2</td>
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<tr>
<td>H373</td>
<td>0.731</td>
<td>0.653</td>
<td>0.679</td>
<td>0.688</td>
<td>8.9</td>
</tr>
<tr>
<td>H374</td>
<td>0.175</td>
<td>0.160</td>
<td>0.167</td>
<td>0.167</td>
<td>9.4</td>
</tr>
<tr>
<td>H375</td>
<td>0.318</td>
<td>0.356</td>
<td>0.324</td>
<td>0.333</td>
<td>7.8</td>
</tr>
<tr>
<td>H377</td>
<td>0.995</td>
<td>1.042</td>
<td>0.962</td>
<td>1.000</td>
<td>5.5</td>
</tr>
</tbody>
</table>

REFERENCES