1. INTENDED USE

The kit has been designed for the quantitative determination of Hepatitis B e-antigen IgG (HBeAb IgG) in human serum. The method can be used for samples over the range of 0-400 index/ml. The test has to be performed on the Maglumi fully auto analyzer (including Maglumi 1000, Maglumi 2000, Maglumi 2000 plus).

2. SUMMARY AND EXPLANATION OF THE TEST

HBV is transmitted through infected body fluids, including blood, semen, and vaginal fluids (including menstrual blood). It also can be transmitted from a pregnant woman to her child at or near the time of birth. Hepatitis B surface antigen (HBeAg IgG) is one of the most frequently performed tests for HBV. This HBV antigen is the earliest indicator of an active hepatitis B infection. This antigen may be present before symptoms of an HBV infection are present. If this antigen level remains high for more than 6 months, then you will probably become a carrier of HBV, meaning you can transmit it to others throughout your life. Hepatitis B surface antibody (HBeAb) is also one of the most common tests for HBV. Usually this antibody appears about 4 weeks after HBeAg IgG disappears and means that the infection is at the end of its active stage and you cannot pass the virus to others (you are no longer contagious). This antibody also protects you from getting HBV again in the future. The test is done to determine the need for vaccination; the antibody will be present after receiving the HBV vaccine series, showing that you have protection (immunity) from the virus. Occasionally your test may show that you have both the HBeAg and HBeAb IgG antibodies; in this case, you are still contagious. Hepatitis B core antigen (HBcAg). Currently, there is no test to find this antigen. Hepatitis B core antibody (HBcAb) is an antibody to the hepatitis B core antigen. This antibody appears about 1 month after an active HBV infection. It can be found in people who had an infection in the past and in those with long-term (chronic) HBV. It usually is present for life. Hepatitis B core antibody IgM (HBcAbIgM) is also an antibody to the hepatitis B core antigen. It shows a recent infection in the last 6 months. Hepatitis B e-antigen (HBeAg) is an HBV protein that is only present during an active HBV infection. This test determines how contagious you are. Testing for this antigen can also be used to monitor the effectiveness of treatment for HBV. Hepatitis B e-antibody (HBeAb) shows that the active stage of the HBV infection is almost over and your risk of being contagious is greatly reduced. HBeAg is usually present during chronic HBV infections. HBV DNA testing finds genetic material (DNA) from the hepatitis B virus. Currently, quantitative HBV DNA tests are done. A high HBV DNA level means that the virus is multiplying in your body and you are very contagious. If you have a chronic HBV infection, an elevated viral DNA level means you are at an increased risk for chronic hepatitis and may want to consider treatment with medications. Testing for HBV DNA also is important to monitor the effectiveness of treatment for chronic HBV infection. HBV DNA testing is a more sensitive test than HBeAg IgG (above) for detecting HBV in the blood.

3. PRINCIPLE OF THE TEST

Antibody competitive sandwich immunoluminometric assay:

Use anti-HBe monoclonal antibody to label ABEI, another monoclonal antibody to label FITC. Add limited free HbeAg. ABEI Label and FITC Label are competed to conjugate HBeAg with Sample, Calibrator or Control, and diluted human serum, forming antibody-antigen complexes; Add nano magnetic microbeads coated with sheep anti-FITC then mixed thoroughly, after sediment in a magnetic field, decant the supernatant, then cycle washing for 1 time. Subsequently, the starter, reagents are added and a flash chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier as RLU within 3 seconds and is proportional to the concentration of HBeAb IgG present in controls or samples.

4. KIT COMPONENTS

4.1 Material supplies

<table>
<thead>
<tr>
<th>Item</th>
<th>Quantity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaNO₃, coated with sheep anti-FITC polyclonal antibody.</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Calibrator low:</td>
<td>2.5ml</td>
</tr>
<tr>
<td>Calibrator high:</td>
<td>2.5ml</td>
</tr>
<tr>
<td>ABEI Label:</td>
<td>6.5ml</td>
</tr>
<tr>
<td>ABEI Label: anti-HBe monoclonal antibody labeled ABEI, containing BSA, 0.2%NaNO₃.</td>
<td>6.5ml</td>
</tr>
<tr>
<td>FITC Label: anti-HBe monoclonal antibody labeled FITC, containing BSA, 0.2%NaNO₃.</td>
<td>6.5ml</td>
</tr>
<tr>
<td>Free HBeAg</td>
<td>6.0ml</td>
</tr>
<tr>
<td>Diluent</td>
<td>25ml</td>
</tr>
</tbody>
</table>
4.2 Preparation of the Reagent Integral
Before the sealing is removed, gentle and careful horizontal shaking of the Reagent Integral is essential (avoid foam formation). Remove the sealing and turn the small wheal of the magnetic microbeads compartment to and fro, until the colour of the suspension has changed into brown. Place the Integral into the reagent area and let it stand there for 30 mins. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange Nano Magnetic Microbeads from different reagents!

4.3 Storage of the Reagents Integral
- Sealed: Stored at 2-8°C until the expiry date.
- Opened: Stable for 4 weeks. After this period, it is still possible to keep on using the Reagent Integral provided that the controls are found within the expected ranges.
- Keep upright for storage.
- Keep away from direct sunlight.

5. Origin of Calibrators.
Calibrators in the Reagent Kit are from Fitzgerald.

6. Calibration
6.1 2 point recalibration
Via the measurement of calibrators, the predefined master curve is adjusted (recalibrated) to a new, instrument-specific measurement level with each calibration.

6.2 Frequency of Recalibration
- After each exchange of lot (Reagent Integral or Starter Reagent).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Maglumi Fully Auto analyzer.
- If controls are beyond the expected range.
- Room temperature changes exceed 5°C (recommendation).

7. Sample Collection, Material and Storage
- Collect samples using standard procedures.
- Sample material: serum
- Store at 2-8°C: 24 hours.
- For longer storage periods: freeze to below -20°C.
- Avoid repeated freezing and thawing cycles.
- Stored samples should be thoroughly mixed prior to use (Vortex mixer).
- Vacuum tubes (a) Blank tubes are recommended type for collecting samples.
(b) If plasma sample is needed, EDTA tube is in conformity has no effect on the results RLU.
(c) Liquaemin Sodium tube is found to increase the sample RLU and cause test results deviation.
(d) Please ask SNIBE for advice if special additive must be used in the sample blood.

8. WARNING AND PRECAUTIONS FOR USERS
- For use in in-VITRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- Do not pipette by mouth.
- Do not eat, drink, smoke or apply cosmetics in the assay laboratory.
- Avoid microbial contamination of reagents during pipetting by using disposable pipette tips.
- Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands at the end of each assay.
- Avoid splashing or forming an aerosol. Any reagent spills should be washed with a 5% hypochlorite solution and disposed of as though potentially infectious.
- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents. They should therefore be disposed of in accordance with the prevailing regulations and guidelines of the agencies holding jurisdiction over the laboratory, and the regulations of each country. Disposable materials must be incinerated; liquid waste must be decontaminated with sodium hypochlorite at a final concentration of 5% for at least half an hour. Any materials to be reused must be autoclaved using an overkill approach (USP 24, 2000, p. 2143). A minimum of one hour at 121°C is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- The calibrators in this kit are prepared from bovine serum products. However, because no test method can offer complete assurance that HIV, Hepatitis B Virus or other infectious agents are absent, these reagents should be considered a potential biohazard and handled with the same precautions as applied to any serum or plasma specimen.

9. Test Procedure
To ensure proper test performance, strictly adhere to the operating instructions of the Maglumi Fully Auto analyzer. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

<table>
<thead>
<tr>
<th>Auto-dilution</th>
<th>Sample, Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>+20μl</td>
<td>Sample, calibrator or control</td>
</tr>
<tr>
<td>+10μl</td>
<td>Free HBeAg</td>
</tr>
<tr>
<td>+40μl</td>
<td>ABEI Label</td>
</tr>
<tr>
<td>+40μl</td>
<td>FITC Label</td>
</tr>
<tr>
<td>+20μl</td>
<td>Nano magnetic microbeads</td>
</tr>
</tbody>
</table>

10. Quality Control
- Observe quality control guidelines for medical laboratories.
- Use suitable controls for in-house quality control.

11 Results
11.1 Calculation of Results
- The analyzer automatically calculates the HBeAg IgG concentration in each sample by means of a calibration curve which is generated by a 2-point calibration master curve procedure. The results are expressed in index/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.
- Results need NOT to multiply dilution rate.

11.2 Interpretation of Results
- Reference values: <100 index/ml.
- Results may differ between laboratories due to variations in population and test method. Each laboratory should establish its own reference range.

12. Limitations of the procedure
12.1 A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Procedural directions must be followed exactly and careful technique must be used to obtain valid results. Any modification of the procedure is likely to alter the results. Bacterial contamination or repeated freeze-thaw cycles may affect the test results.

13. Performance Characteristics
13.1 Accuracy
Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diulents, and measure its diluted concentration for 10 times. Then calculate the recovery of measured concentration and expected concentration. The recovery should be within 90% - 110%.

13.2 Precision
Intra-assay coefficient of variation was evaluated on Calibrator High repeatedly measured 10 times in the same assay, calculating their coefficient of variation, the results should ≤10%. Inter-assay coefficient of variation was evaluated on three batches of kit, repeatedly measured 10 times of Calibrator High, calculating three batches of kit for Calibrator High between the measured values of the coefficients of variation, the results should ≤15%.

13.3 Sensitivity
* In case of lacking of Diluent, please order more from SNIBE. This diluent is special buffer for this assay.

All reagents are provided ready-to-use.

*Please prepare 0.9% sodium chloride solution in case of insufficient diluents.

Accessories required but not provided
Maglumi Reaction module
Maglumi - Starter kit 1+2
Maglumi Light check
Maglumi Wash /System Liquid

<table>
<thead>
<tr>
<th>121</th>
<th>≤ 10%</th>
</tr>
</thead>
<tbody>
<tr>
<td>15%</td>
<td></td>
</tr>
</tbody>
</table>

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The sensitivity of the assay defined as the concentration of HBeAb IgG equivalent to the mean RLU of 20 replicates of the zero standard plus two standard deviations corresponding to the concentration from the standard curve. The sensitivity is typically less than 5.0 index/ml.

13.4 Specificity
The specificity of the HBeAb assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes. When HBsAb=334.37 index/ml, the detection of HBeAb is less than <10.0 index/ml.

13.5 Linearity
Conduct a logarithmic transform to the RLU value and concentration value of 6 standards. After a double logarithmic fitting, the absolute value of its linearity should exceed 0.9800.

14. References
8. Abdel-Ghaffar AY. Effect of schistosomiasis on cellular immunity. MD thesis Cairo, Ain Shams University, Faculty of Medicine, 1990.