1. INTENDED USE

The kit has been designed for the quantitative determination of EBV VCA IgG in human serum. The method can be used for samples over the range of 0-30 AU/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

Epstein-Barr virus (EBV) is the etiologic agent of infectious mononucleosis (IM) and is implicated in Burkitt’s lymphoma (BL), nasopharyngeal carcinoma (NPC) and X-linked lymphoproliferative syndrome (XLP). EBV is a human herpesvirus pathogenic for man. Since it is ubiquitous, it infects nearly 95% of individuals worldwide by adulthood. The DNA of EBV is composed of a double strand molecule of approximately 172 kbases in length.

The major route of transmission of EBV is through oral contact. Replication of EBV occurs in the oropharyngeal epithelium and results in the release of virions from infected B lymphocytes, with consequent shedding of infectious particles into the saliva. During childhood, primary infection with EBV is often asymptomatic. Acquisition of the virus during adolescence through adulthood results in infectious mononucleosis in the majority of persons. After primary infection, EBV remains latent for life.

Diagnosis of infectious mononucleosis is based upon clinical manifestations (which generally include sore throat, fever, lymphadenopathy, and malaise) in conjunction with haematological evidence for lymphocytosis and serological evidence for the presence of heterophile antibody and/or antibodies to EBV specific proteins.

Clinical manifestations similar to infectious mononucleosis can also be induced by a number of other pathogenic infectious agents including cytomegalovirus, Toxoplasma gondii, hepatitis viruses, human immunodeficiency virus (HIV), and others. The term mononucleosis syndrome is often applied until the specific etiologic agent is identified. Confirmation of an acute diagnosis of EBV infectious mononucleosis is generally sought by a positive heterophile antibody test (agglutination by patient’s serum with horse or sheep red blood cells). However, difficulties in diagnosis arise when the heterophile test is negative or when clinical manifestations are atypical.

Heterophile-negative infectious mononucleosis has been demonstrated in 10 to 20% of adults with an even greater percent- age in children with acute infectious mononucleosis infections. For these individuals, diagnosis of infectious mononucleosis may be confirmed by identification of antibodies to specific EBV protein antigens which include viral capsid antigen (VCA) and early antigen-diffuse [EA(D)]. The presence of IgM antibody to VCA is instrumental for diagnosis of acute infectious mononucleosis. However, verification should be sought by assaying for other corroborating antibodies – such as EA(D) IgG or predominance of EBNA-1 IgG or EBNA-1 IgM antibody – and with additional clinical information. Serological heterophile- negative samples demonstrating EBV VCA IgM and transient levels of EA(D) IgG antibodies have been considered diagnostic for acute infectious mononucleosis.

Serologic testing for EBV infection is possible because characteristic time-dependent antibody responses occur. A cur- rent primary EBV infection is defined serologically by the early appearance of circulating VCA IgM and their subsequent decrease to non-detectable levels. Almost concurrently, an increase in VCA IgG appears. Most (>80%) symptomatic infectious mononucleosis patients show near-peak antibody levels of VCA IgG and IgM when first examined. VCA IgM antibodies usually disappear in two to three months of the onset of disease, while IgG antibodies persist indefinitely in normal persons. Most patients transiently develop antibodies to EA(D), but IgG antibodies to Epstein-Barr nuclear antigen (EBNA) appear in the circulation several weeks or months after the onset of disease and persist for years or even life. In symptomatic infectious mononucleosis patients, detection of IgG antibodies to EBNA, when detected in concert with VCA IgM and IgG antibodies, is useful in discerning early convalescent stages from acute stages of infectious mononucleosis. A rise in EBNA IgG level in infectious mononucleosis patients may be indicative of progression from early to later stages of convalescence. A rise in VCA IgG level is indicative of an acute stage of infection, while a rise in VCA IgM levels may be indicative of progression from an early to an acute stage of infection. Similarly, a drop in VCA IgM level may be indicative of progression from an acute to a waning stage of infection. The presence of EBNA IgG antibodies in healthy individuals indicates past immunological exposure to EBV; that of VCA IgG antibodies indicates immunological exposure to EBV either as silent primary infection or past exposure.

Because of the complex relationship that exists between host reaction to EBV and clinical manifestations, tracking of EBV antibody patterns may assist in diagnosis of EBV infection. Individual levels of specific antibodies...
are not necessarily indicative of disease state but can be of diagnostic significance when tracked as an antibody response profile. Antibody response profiles for the different EBV antigens demonstrate a characteristic pattern for silent primary or persistent latent EBV infections, as well as for each of the EBV-associated diseases.

3. PRINCIPLE OF THE TEST
Sandwich immunoluminescent assay:
anti-IgG monoclonal antibody is used to label ABEI, and use purified EBV VCA antigen to coat nano magnetic microbeads. Autocontrol-sample, Calibrators, or Control, Buffer and nano magnetic microbeads coated with EBV VCA antigens are mixed thoroughly and incubated at 37°C and cycle washing for 1 time. Then add ABEI Label, incubation and form a sandwich, then washing for the 2nd time. Subsequently, the 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 EBV VCA IgG present in controls or samples.

4. KIT COMPONENTS
4.1 Material supplies

<table>
<thead>
<tr>
<th>Reagent Integral for 100 determinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN3, coated with purified EBV VCA antigen</td>
</tr>
<tr>
<td>Calibrator low</td>
</tr>
<tr>
<td>Calibrator high</td>
</tr>
<tr>
<td>ABEI label: anti-IgG monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN3</td>
</tr>
<tr>
<td>Buffer, containing BSA, 0.2%NaN3</td>
</tr>
<tr>
<td>Diluent</td>
</tr>
</tbody>
</table>

All reagents are provided ready-to-use.

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 wheel 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 aggregated 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.
Biological root: recombinant protein.No HBsAg, anti-HCV, and anti-HIV is found.

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 Reagents).
- 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 conformed has no effect on the results RLU's.
  (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 IV+IVTRO diagnostic procedures only.
- Do not interchange reagents from different lots. Do not use kit components beyond their labeled expiry date.
- 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>Autodilution</th>
<th>1:11 T</th>
<th>20μl</th>
<th>+200μl</th>
<th>Sample Diluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>20μl</td>
<td>+100μl</td>
<td>+20μl</td>
<td>Buffer Nano magnetic microbeads coated with purified EBV VCA antigen.</td>
<td></td>
</tr>
<tr>
<td>10 min</td>
<td>Incubation</td>
<td>400μl each time</td>
<td>Cycle washing</td>
<td></td>
</tr>
<tr>
<td>+100μl</td>
<td>ABEI Label</td>
<td>10 min</td>
<td>Incubation</td>
<td></td>
</tr>
<tr>
<td>400μl each time</td>
<td>Cycle washing</td>
<td>3 s</td>
<td>Measurement</td>
<td></td>
</tr>
</tbody>
</table>

* Do not interchange magnetic microbeads from different lots

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 EBV VCA 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 AU/ml. For further information please refer to the Maglumi Fully Auto Operator’s Manual.

11.2 Interpretation of Results
- Reference values: < 4 AU/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 Use EBV VCA IgG value as a kind of auxiliary material for other testing data when in diagnosis. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions. A skillful technique and strict adherence to the instructions are necessary to obtain reliable results. Bacterial contamination of samples or repeated freeze-thaw cycles may affect the test results. Assay results should be utilized in conjunction with other clinical and laboratory data to assist the clinician in making individual patient management decisions.

13. Performance Characteristics
13.1 Accuracy
Consider High Calibrator of known concentration as a sample, dilute it by 1:2 ratio with diluent, 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
The sensitivity is defined as the concentration of EBV VCA 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 0.25 AU/ml.

13.4 Specificity
The specificity of the EBV VCA IgG assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analytes.
When EBV NA IgG or EBV EA IgG=30AU/ml the detection results of EBV VCA IgG <1 AU/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
2. Essentials of Anatomic Pathology - Page 3-5 by Liang Cheng, David G Bostwick - Medical - 2002 - 920 pages