MAGLUMI EBV VCA IgA (CLIA)

REF 130215005M

Σ 100

EC REP

Shenzhen New Industries Biomedical Engineering Co., Ltd
4/F,Wearnes Tech Bldg,
Science & Industry Park,
Nanshan,Shenzhen,518057CHINA
Tel. + 86-755-86028224
Fax.+ 86-755-26654850

Lotus Global Co., Ltd
15 Alexandra Road
London
UK
NW8 0DP
Tel. + 44-20-75868010
Fax.+ 44-20-79006187

CE

FOR PROFESSIONAL USE ONLY
Store at 2-8 °C

COMPLETELY READ THE INSTRUCTIONS BEFORE PROCEEDING

en

SYMBOLS EXPLANATIONS

EC REP	Authorized Representative in the European community
	Manufacturer
	Consult instructions for use
CONT	Contents of kit
IVD	In vitro diagnostic medical device
LOT	Batch code
REF	Catalogue number
	Use by
8°C 2°C	Temperature limitation (store at 2-8 °C)
Σ	Sufficient for
	Keep away from sunlight
	Keep upright for storage

INTENDED USE

The kit has been designed for the qualitative determination of EBV VCA IgA in human serum.
The test has to be performed on the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI (Including Maglumi 600,Maglumi 1000,Maglumi 1000 Plus, Maglumi 2000,Maglumi 2000 Plus,Maglumi 3000 and Maglumi 4000).

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 lympho -proliferative syndrome (XLP). EBV is one 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, lympha -denopathy, 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 demon -strating 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 current 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.

PRINCIPLE OF THE TEST

Indirect immunoluminometric assay:

Anti-IgA monoclonal antibody is used to label ABEI, and use purified EBV VCA antigen to coat nano magnetic microbeads. Sample, Calibrator or Control with Buffer（goat Anti-human IgM, goat Anti-human IgG） and nano magnetic microbeads coated with EBV VCA antigen are mixed thoroughly and incubated at 37℃ and cycle washing for 1 time. Then add ABEI Label, incubation and form a sandwich, then washing for the 2nd 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 EBV VCA IgA present in samples.

KIT COMPONENTS

Material Supplies

Reagent Integral for 100 determinations	
Nano magnetic microbeads: TRIS buffer, 1.2%(W/V), 0.2%NaN_3, coated with purified EBV VCA antigen	2.5ml
Calibrator low: bovine serum,0.2%NaN_3	2.5ml
Calibrator high: bovine serum,0.2%NaN_3	2.5ml
ABEI label: anti-IgA monoclonal antibody labeled ABEI, containing BSA, 0.2%NaN_3	12.5ml
Buffer: Containing BSA, 0.2%NaN_3. Goat anti-Human IgG, Goat anti-Human IgM	22.5ml
All reagents are provided ready-to-use.	

Reagent Vials in kit box	
Internal Quality Control: containing BSA, 0.2%NaN_3. (target value refer to Quality Control Information date sheet)	2.0ml

Internal quality control is only applicable with MAGLUMI system. Instructions for use and target value refer to Quality Control Information date sheet. User needs to judge results with their own standards and knowledge.

Accessories Required But Not Provided

MAGLUMI Reaction Module	REF: 630003
MAGLUMI Starter 1+2	REF: 130299004M
MAGLUMI Wash Concentrate	REF: 130299005M
MAGLUMI Light Check	REF: 130299006M

Please order accessories from SNIBE or our representative.

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 min. During this time, the magnetic microbeads are automatically agitated and completely resuspended.

Do not interchange integral component from different reagents or lots!

Storage and Stability

- Sealed: Stored at 2-8℃ until the expiry date.
- Opened: Stable for 4 weeks. To ensure the best kit performance, it is recommended to place opened kits in the refrigerator if it's not going to be used on board during the next 12 hours.
-  Keep upright for storage.
-  Keep away from sunlight.

CALIBRATION AND TRACEABILITY

1) Traceability

To perform an accurate calibration, we have provided the test calibrators standardized against the SNIBE internal reference substance.

Calibrators in the Reagent Kit are from BIODESIGN

2) 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.

3) Frequency of Recalibration

- After each exchange of lots (Reagent Integral or Starter Reagents).
- Every week and/or each time a new Integral is used (recommendation).
- After each servicing of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI.
- If controls are beyond the expected range.
- The room temperature has changed more than 5 ℃ (recommendation).

SPECIMEN COLLECTION AND PREPARATION

Sample material: serum

Collect 5.0ml venous blood into Blood Collection Tube (Tube without anticoagulant or coagulant, Anticoagulation tube with EDTA-K_2 or EDTA-Na_4 can be used. Anticoagulation tube with heparin sodium is not recommended).

Standing at room temperature，centrifuging, separating serum part.

The serum sample is stable for up to 12 hours at 2-8℃. If preserved for more than 12 hours, please packed, -20 ℃ can be stored for 30 days.
Avoid repeated freezing and thawing, the serum sample can be only frozen and thawed two times. Stored samples should be thoroughly mixed prior to use (Vortex mixer).
Please ask local representative of SNIBE for more details if you have any doubt.

Vacuum Tubes

(a) Blank tubes are recommended type for collecting samples.
(b) Please ask SNIBE for advice if special additive must be used in sample collecting.

Specimen Conditions

- Do not use specimens with the following conditions:
  (a) heat-inactivated specimens;
  (b) Cadaver specimens or body fluids other than human serum;
  (c) Obvious microbial contamination.
- Use caution when handling patient specimens to prevent cross contamination. Use of disposable pipettes or pipette tips is recommended.
- Inspect all samples for bubbles. Remove bubbles with an applicator stick prior to analysis. Use a new applicator stick for each sample to prevent cross contamination.
- Serum specimens should be free of fibrin, red blood cells or other particulate matter.
- Ensure that complete clot formation in serum specimens has taken place prior to centrifugation. Some specimens, especially those from patients receiving anticoagulant or thrombolytic therapy, may exhibit increased clotting time. If the specimen is centrifuged before a complete clot forms, the presence of fibrin may cause erroneous results.

Preparation for Analysis

- Patient specimens with a cloudy or turbid appearance must be centrifuged prior to testing. Following centrifugation, avoid the lipid layer (if present) when pipetting the specimen into a sample cup or secondary tube.
- Specimens must be mixed **thoroughly** after thawing by **low** speed vortexing or by gently inverting, and centrifuged prior to use to remove red blood cells or particulate matter to ensure consistency in the results. Multiple freeze-thaw cycles of specimens should be avoided.
- All samples (patient specimens or controls) should be tested within 3 hours of being placed on board the MAGLUMI System. Refer to the SNIBE service for a more detailed discussion of onboard sample storage constraints.

Storage

- If testing will be delayed for more than 8 hours, remove serum from the serum separator, red blood cells or clot. Specimens removed from the separator gel, cells or clot may be stored up to 12 hours at 2-8°C.
- Specimens can be stored up to 30 days frozen at -20°C or colder.

Shipping

- Before shipping specimens, it is recommended that specimens be removed from the serum separator, red blood cells or clot. When shipped, specimens must be packaged and labeled in compliance with applicable state, federal and international regulations covering the transport of clinical specimens and infectious substances. Specimens must be shipped frozen (dry ice). Do not exceed the storage time limitations identified in this section of the package insert.

WARNING AND PRECAUTIONS FOR USERS

IVD

- For use in *IN-VITRO* diagnostic procedures only.
- Package insert instructions must be carefully followed. Reliability of assay results cannot be guaranteed if there are any deviations from the instructions in this package insert.

Safety Precautions

CAUTION: This product requires the handling of human specimens.

- 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.
- 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. A minimum of one hour at 121℃ is usually considered adequate, though the users must check the effectiveness of their decontamination cycle by initially validating it and routinely using biological indicators.
- It is recommended that all human sourced materials be considered potentially infectious and handled in accordance with the OSHA Standard on Bloodborne Pathogens 13. Biosafety Level 214 or other appropriate biosafety practices should be used for materials that contain or are suspected of containing infectious agents.
- This product contains Sodium Azide; this material and its container must be disposed of in a safe way.
- Safety data sheets are available on request.

Handling Precautions

- Do not use reagent kits beyond the expiration date.
- Do not mix reagents from different reagent kits.
- Prior to loading the Reagent Kit on the system for the first time, the microbeads requires mixing to re-suspend microbeads that have settled during shipment.
- For microbeads mixing instructions, refer to the KIT COMPONENTS, Preparation of the Reagent Integral section of this package insert.
- To avoid contamination, wear clean gloves when operating with a reagent kit and sample.
- Over time, residual liquids may dry on the kit surface, please pay attention the silicon film still exists on the surface of the kit.
- For a detailed discussion of handling precautions during system operation, refer to the SNIBE service information.

TEST PROCEDURE

To ensure proper test performance, strictly adhere to the operating instructions of the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI. Each test parameter is identified via a RFID tag on the Reagent Integral. For further information please refer to the Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

10μl +200μl	Sample, calibrator Buffer
10 min	Incubation
+20μl	Nano magnetic microbeads
10 min	Incubation

400µl	Cycle washing
+100µl	ABEI Label
10 min	Incubation
400µl	Cycle washing
3 s	Measurement

* Do not interchange magnetic microbeads from different lots.

QUALITY CONTROL

- Observe quality control guidelines for medical laboratories
- Use suitable controls for in-house quality control. Controls should be run at least once every 24 hours when the test is in use, once per reagent kit and after every calibration. The control intervals should be adapted to each laboratory's individual requirements. Values obtained should fall within the defined ranges. Each laboratory should establish guidelines for corrective measures to be taken if values fall outside the range.

LIMITATIONS OF THE PROCEDURE

1) Limitations

Use EBV VCA IgA 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.

2) Interfering Substances

No interference with test results is seen by concentrations of bilirubin ⩽ 0.4mg/ml, haemoglobin ⩽ 10mg/ml，Triglycerides ⩽ 20mg/ml.

3) HAMA

Patient samples containing human anti-mouse antibodies (HAMA) may give falsely elevated or decreased values. Although HAMA-neutralizing agents are added, extremely high HAMA serum concentrations may occasionally influence results.

4) High-Dose Hook

No high dose hook effect was observed when samples containing up to 2000 AU/ml.

RESULTS

1) Calculation of Results

1. The analyzer automatically calculates the EBV VCA Ig A 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 Fully-auto chemiluminescence immunoassay (CLIA) analyzer MAGLUMI Operating Instructions.

2) Interpretation of Results

Results obtained with the MAGLUMI EBV VCA IgA assay can be interpreted as follows:

2. Non-reactive: A result less than 4.0 AU/ml (< 4.0 AU/ml) is considered to be negative.
3. Reactive: A result greater than or equal to 4.0 AU/ml is (≥ 4.0 AU/ml) considered to be positive.

Since there is no EBV VCA IgA international standard material yet, different IVD manufacturer have different traceability chain. Therefore results from assays of other manufacturers cannot be used interchangeably.

PERFORMANCE CHARACTERISTICS

1) Precision

Intra-assay coefficient of variation was evaluated on 3 different levels of control serum repeatedly measured 20 times in the same run, calculating the coefficient of variation.

Intra-assay precision			
Control	Mean(AU/ml)	SD(AU/ml)	CV%
Level 1	2.01	0.10	4.76%
Level 2	7.35	0.34	4.61%
Level 3	19.57	0.83	4.25%

Inter-assay coefficient of variation was evaluated on three batches of kits. Repeatedly measured 3 different levels of control serum 21 times, calculating the coefficient of variation.

Inter-assay precision			
Control	Mean(AU/ml)	SD(AU/ml)	CV%
Level 1	2.27	0.22	9.71%
Level 2	7.61	0.70	9.23%
Level 3	20.04	1.83	9.15%

2) Analytical Sensitivity

The sensitivity is defined as the concentration of EBV VCA Ig A 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.

3) Specificity

The specificity of the EBV VCA IgA assay system was assessed by measuring the apparent response of the assay to various potentially cross reactive analysts.

When CMV IgG, CMV IgM, Rubella IgG, Rubella IgM, Toxo IgG, Toxo IgM, HSV-1/2 IgG, HSV-1/2 IgM, EBV EA IgA separately reach a concentration of 30 AU/ml, measured EBV VCA IgA is negative. No cross reaction with IgG or IgM of HAV, HBV, HCV, HIV, Syphilis and other EBV antibody. The ELISA diagnosed RF or ANA positive, which is non EBV infected sample, this reagent's determination results show negative.

4) Recovery

Consider calibrator high of known concentration as a sample, dilute it by 1:2 ratio with diluents, 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%.

Expected	Mean Measuring	Recovery
16.91 AU/ml	17.45 AU/ml	103 %

REFERENCES

1. Maeda E, Akahane M, Kiryu S, et al. (January 2009). "Spectrum of Epstein-Barr virus-related diseases: a pictorial review". Jpn J Radiol 27 (1): 4–19.
2. Toussirot E, Roudier J (October 2008). "Epstein-Barr virus in autoimmune diseases". Best Practice & Research. Clinical Rheumatology 22 (5): 883–96.
3. Dreyfus DH (December 2011). "Autoimmune disease: A role for new anti-viral therapies?". Autoimmunity Reviews 11 (2): 88–97.
4. Ascherio A, Munger KL (September 2010). "Epstein-Barr virus infection and multiple sclerosis: a review". Journal of Neuroimmune Pharmacology : the Official Journal of the Society on NeuroImmune Pharmacology 5 (3): 271–7.
5. M. A. Epstein, B. G. Achong, and Y. M. Barr: Virus particles in cultured lymphoblasts from Burkitt's lymphoma. The Lancet, March 28, 1964, 1: 702–703
6. Young, LS (2009). Desk Encyclopedia of Human and Medical Virology. Boston: Academic Press. pp. 532–533.