EBNA IgG (Epstein Barr Virus Nuclear Antigen)

Purpose

This procedure provides instructions for performing EBNA IgG (Epstein Barr Virus Nuclear Antigen) on the DIASORIN LIAISON.

Policy Statements

This procedure applies to all laboratory technical staff responsible for performing EBNA IgG testing on the DiaSorin Liaison.

Principle

The LIAISON® EBNA IgG assay uses chemiluminescent immunoassay (CLIA) technology on the LIAISON® Analyzer for the qualitative determination of specific IgG antibodies to Epstein-Barr virus (EBV) nuclear antigen synthetic peptide (EBNA-1) in human serum. When performed in conjunction with other EBV markers, this assay can be used as an aid in the clinical laboratory diagnosis of Epstein-Barr Viral Syndrome in patients with signs and symptoms of EBV infection such as infectious mononucleosis.

The method for qualititative determination of specific IgG to EBV nuclear antigen (EBNA) is an indirect chemiluminescence immunoassay (CLIA). All assay steps (with the exception of magnetic particle resuspension) and incubations are performed by the LIAISON® Analyzer. The principal components of the test are magnetic particles (solid phase) coated with EBNA-1 synthetic peptide and a conjugate of mouse monoclonal antibody to human IgG linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, EBNA IgG antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with EBNA IgG antibodies that are already bound to the solid phase. After each incubation, unbound material is removed with a wash cycle. Subsequently, the starter reagents are added and a flash chemiluminescence reaction is induced. The light signal, and hence the amount of isoluminol-antibody conjugate, is measured by a photomultiplier as relative light units (RLU) and is indicative of the presence of EBV EBNA IgG antibodies present in calibrators, samples or controls.

Clinical Significance

Epstein-Barr virus (EBV) is responsible for infectious mononucleosis (IM) and is implicated in Burkitt's lymphoma and nasopharyngeal carcinoma. Diagnosis of IM is based upon clinical manifestations that generally include sore throat, fever, lymphadenopathy, and malaise in conjunction with hematological evidence for lymphocytosis and serological evidence for the presence of heterophile antibody and/or EBV antibodies to specific proteins. Clinical manifestations similar to IM 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 IM 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 IM has been demonstrated in 10 to 20% of adults with an even greater percentage in children with acute IM infections. For these individuals, IM diagnosis 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 VCA IgM antibody usually suffices for diagnosis of IM. However, verification should be sought with additional clinically relevant information (4). Serologic testing for EBV infection is possible because characteristic time-dependent antibody responses occur. Most (> 80%) symptomatic IM patients show near-peak antibody levels of VCA IgG and IgM when first examined. VCA IgM antibodies usually disappear in 2-3 months while IgG antibodies persist indefinitely. Most patients transiently develop antibodies to EA(D), but IgG antibodies against Epstein-Barr Nuclear Antigen (EBNA) appear several weeks or months after the onset of disease and persist for years or even life. In symptomatic IM 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 IM infection. A rise in EBNA IgG level in IM patients may be indicative of progression from early to later stages of convalescence.

The presence of EBNA IgG antibodies in healthy individuals indicates past immunological exposure to EBV. Because of the complex relationship that exists between the EBV virus/host reaction and clinical manifestation, 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.

Instrument

DiaSorin LIAISON®, DiaSorin, Inc. Stillwater, MN
Sunquest Method Code: **LIAS**

Sunquest Test Code

EBNA: EBV Nuclear antigen, not an orderable test individually. EBNA is a member of the panel:
EBVS: Epstein Barr antibody serology

Materials

Reagents	Supplies	Equipment
LIAISON® EBNA IgG (310520) Integral, supplied ready to use, containing magnetic particles, calibrators, diluent and conjugate. Materials required but not provided: LIAISON® Module (part # 319130) LIAISON® Cleaning Kit (part # 310990) LIAISON® Starter Kit (part # 319102) LIAISON® Light Check (part # 319101) LIAISON® Wash/System Fluid (part # 319100) LIAISON® Waste Bags (part # 9450003) LIAISON® EBNA IgG Controls (negative, positive)	Transfer Pipet capable of delivering 250 µL Glass or polypropylene sample tubes	DiaSorin Liaison System

Reagent Integral Preparation

1.	Magnetic particles must be completely resuspended before the Integral is placed on the instrument.
2.	Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the color of the suspension has changed to brown. Gentle and careful side-to-side mixing may assist in the suspension of the magnetic particles (avoid foam formation).
3.	Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended
4.	Remove the seal from each container of the Reagent Integral and turn the thumb wheel at the bottom of the magnetic particle container back and forth until the suspension turns brown.
5.	Carefully wipe the surface of each septum to remove residual liquid
6.	Place the Reagent Integral into the reagent area of the Analyzer with the bar code label facing left and let it stand for 30 minutes before use. The Analyzer automatically stirs and re-suspends the magnetic particles.
7.	Repeat as necessary until the magnetic particles are completely resuspended.
8.	Visually inspect the reagents, calibrators in particular (position two and three following the magnetic particle vial), to ensure there is no foaming present before using the Integral. If foam is present after resuspension of the magnetic particles, place the Integral on the instrument and allow the foam to dissipate. The Integral is ready to use once the foam has dissipated and the integral has remained onboard and mixing.
9.	Follow the Analyzer Operator's Manual to load the specimens and start the run.

Reagent Integral Storage and Stability:

- Upon receipt, the Reagent Integral must be stored in an upright position to facilitate resuspension of magnetic particles.
- Stored sealed, the reagents are stable at 2-8°C up to the expiration date.
- After removing the seals, the Reagent Integral is stable for eight weeks when stored at 2-8°C or on board the LIAISON® Analyzer. Record new expiration date on the integral.
- Do not freeze.
- The Reagent Integral must not be used past the expiration date indicated on the kit and reagent integral labels.

Sample

Serum is the only acceptable specimen for this assay collected aseptically by venipuncture Refer to specimen collection procedures.
Grossly hemolyzed, lipemic, or particulate samples are not recommended

Minimum volume: 250 µL, actual test volume, 20L
Stability: 2-8 °C / 5 days, 45 days at -20 ºC or colder
Do not store in self-defrosting freezer.
Avoid repeated freeze thaw cycles.

Rejection criteria: Unlabeled tube

Preparation:

- Whole blood specimens should be centrifuged as soon as clotted, according to Specimen Processing procedures prior to analysis. See Processing Procedure Manual.
- Clarify samples having particulate matter, turbidity, lipemia, or erythrocyte debris
- Remove air bubbles before testing
- Transfer serum to a properly labeled tube. Minimum labeling includes sample accession ID, and/ or patient name, medical record number, collection date and time.
- If samples are stored frozen, mix thawed samples well before testing.

Special Safety Precautions

- All samples, biological reagents and materials used in the assay must be considered potentially able to transmit infectious agents.
- Specimens should be handled at the BSL 2 level recommended for any potentially infectious human serum or blood specimen.
- 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.
- Some reagents contain sodium azide as a preservative. Flush drains thoroughly with water after disposal.
- 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.

Calibration

Test of assay specific calibrators allows the detected relative light unit (RLU) values to adjust the assigned master curve. Each calibration solution allows four calibrations to be performed. Refer to the Operator's Manual or LIAISON® Quick Guide for calibration instructions.

Recalibration in triplicate is required when

- With each new lot of reagents (Reagent Integral or Starter Reagents).
- Every 14 days.
- After servicing the LIAISON® Analyzer.
- If quality controls are out of your acceptable range.

Calibrator values are stored in the bar codes on the integral label.

Verify new reagent lots before use by testing Liaison EBNA IgG controls, BioRad ToRCH controls, and a weakly positive patient sample (if available). Maintain a rack of previously tested samples for this purpose in the freezer. Document results on the calibration printout.

Comparable results verify the new reagent lot. Discrepant results must be resolved before the reagent can be used for patient testing.

Analytical Measuring Range (AMR)

EBNA IgG is an FDA-cleared/approved in vitro diagnostic assay that reports the qualitative result based on a predefined cut-off value. Verification of AMR or the cut-off value is not required by CAP or CLIA.

Quality Control (For calibration)

LIAISON® EBNA IgG Controls are used for monitoring substantial reagent failure of the LIAISON® EBNA IgG chemiluminescent immunoassay (CLIA).
These controls are not intended to control the assay for serum specimens

- Negative control (0.9 mL x 2 vials) containing a barcode label
- Positive control (0.9 mL x 2 vials) containing a barcode label
- Allow controls to reach room temperature prior to use. Return controls to the refrigerator immediately after each use.

Frequency: Run 2 levels with each calibration curve. Load the bar-coded control vials into the “C” rack on the Liaison, or transfer 220 μL to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability:
Unopened: Store at 2-8°C. Stable until the date on vial. Do not use past the expiration date
Opened: 4 weeks at 2-8°C between uses.

Acceptable ranges: control results within the expected ranges provided on the control vial label validate the test. When control results are outside the expected ranges, the test is invalid and patient results cannot be reported. Assay calibration should be performed if a control failure is observed and controls and samples must be retested.

Quality Control (Daily)

BIORAD®
Liquichek ToRCH Plus Control, Positive PN# 239 (3.0 mL x 3 vials)
Liquichek ToRCH Plus Control, Negative PN# 228 (3.0 mL x 3 vials)
for use with serum specimens

Frequency: Two levels once per day of use. Load the bar-coded control vials into the "K" rack on the Liaison, or transfer 220 µL to a tube. Affix the appropriate bar code label to the tube and place onto the LIAISON®

Stability:
Unopened: Store at –20 to -70°C. Stable until the date on vial. Do not use past the expiration date
Opened: 60 days at 2-8°C tightly capped between uses.

Sunquest Control Names:

- Negative ToRCH = C-TPNC
- Positive ToRCH = C-TPPC

Acceptable ranges: Ranges are current in Sunquest and the instrument. Refer to the Quality Control Procedure for QC exception codes. Do not report patient results until control results are within expected ranges.

Procedure

Refer to the instrument Operating procedure.
Strict adherence to the Operator's Manual ensures proper assay performance. Each test parameter is identified by the bar codes on the Reagent Integral label. In case of malfunction of the bar code reader, the cartridge cannot be read, and the integral cannot be used.

The Analyzer operations are as follows:

1. Dispense calibrators, controls or specimens into the reaction module.
2. Dispense coated magnetic particles.
3. Dispense specimen diluent.
4. Incubate.
5. Wash with Wash/System liquid.
6. Dispense conjugate into the reaction module.
7. Incubate.
8. Wash with Wash/System liquid.
9. Add the Starter Kit and measure the light emitted.

Interpretation/ Results/Alert Values

The Analyzer automatically calculates EBV EBNA IgG antibody concentrations expressed as U/mL and grades the results.

A **cutoff of 20 U/mL** provides the best balance of sensitivity and specificity.
An **equivocal range of 18.0-21.9 U/mL** was applied to the assay to account for normal measurement imprecision.

Results between 18.0 – 21.9 U/mL (***equivocal)*** should be repeat tested. If the result is the same after repeat testing, a second sample should be collected and tested no less than one or two weeks later.

Warning - When a sample result displays the exclamation mark **(!) flag**, the result obtained lies below the assay's signal range. With Software V2.0, the sample should be retested and graded negative if the result is still below the signal range upon retest. With Software V2.2, the sample result is not reported, and the sample must be retested.

Note - *The magnitude of the measured result*, *above the cutoff, is not indicative of the amount of antibody present*.
The accurate distinction of a primary infection from seronegative status or past infection is a key concern of EBV diagnostics. The presence of other EBV serological markers (e.g. VCA IgM, VCA IgG) should be determined to assess the immunological status to infection with EBV. Based on the results of three commonly-used antibody tests (VCA IgG, VCA IgM, EBNA-1 IgG), distinct serological profiles have been described in the medical literature

Condition	VCA IgG	VCA IgM	EBNA-1 IgG
EBV seronegative	-	-	-
Acute infection	+	+	-
Past infection	+	-	+
Indeterminate			
VCA IgG only	+	-	+
VCA IgM only	-	+	-
EBNA IgG only	-	-	+
Convalescent	+	+	+

Dilutions

Do not dilute. See result Reporting.

Reference Intervals

< 18.0 U/mL = Negative
Absence of detectable EBNA IgG antibodies. A negative result generally excludes past EBV infection. If exposure to Epstein-Barr virus is suspected despite a negative finding, a second sample should be collected and tested no less than one to two weeks later.

18.0 to 21.9 U/mL = Equivocal

≥ 22.0 U/mL = Positive
Presence of detectable EBNA IgG antibodies. A positive result is indicative of past infection.

Limitations

1. Do not heat-inactivate sera.
2. The clinical diagnosis must be interpreted with clinical signs and symptoms of the patient. The results from this kit are not by themselves diagnostic and should be considered in association with other clinical data and patient symptoms.
3. Results from immunosuppressed patients should be interpreted with caution. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients, cord blood, neonatal specimens, or infants.
4. Diseases such as cytomegalovirus, toxoplasmosis and hepatitis may cause symptoms similar to infectious mononucleosis and must be excluded before confirmation of diagnosis.
5. The combined use of EBV serological markers and clinical data is recommended when the diagnosis of EBV infection is based on a single serum specimen. A single result cannot be used for diagnosis. Accurate interpretation of EBV infection is based on results of EA(D) IgG, VCA IgM, VCA IgG, EBNA IgG, EBNA IgM and heterophile antibodies.
6. The performance characteristics have not been established for patients with nasopharyngeal carcinoma, Burkitt's lymphoma, EBV-associated lymphadenopathies and other EBV-associated diseases besides EBV-related mononucleosis.
7. Assay performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, and other EBV-associated lymphomas.
8. Assay interference due to circulating antibodies against HIV and Hepatitis A, Hepatitis B and Hepatitis C viruses has not been evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents.

Interferences: assay performance was not affected by

- hemolysis (at 1000 mg/dL hemoglobin)
- lipemia (at 3000 mg/dL triglycerides)
- icterus (at 10 mg/dL bilirubin).

Result Reporting

Review, validate, and tag results and send to Sunquest.
Release results in Sunquest following LIS procedures for OEM. Comments are automatically appended when resulting in OEM or MEM using the LIAS worksheet.

- Results <18.0 U/mL without error messages are reported with the numerical result, and interpreted as Negative. Append the comment “A negative result generally excludes past EBV infection”
- Results between 18.0 – 21.9 U/mL must be repeated prior to reporting and are reported with the numerical result, and interpreted as Equivocal. Append the comment “a second sample should be collected and tested in one or two weeks”
- Results > 21.9 U/mL without error messages are reported with the numerical result, and interpreted as Positive. Append the comment “Presence of detectable EBNA IgG antibodies. A positive result is indicative of past infection.”

Alternate Methods

- When test performance does not meet quality standards, consult the technical specialist or Medical Director, and refer testing to Mayo Medical Laboratory.
- Order test 84421, Epstein Barr virus Antibody Profile, and submit 1.0 mL of serum, 0.6 mL minimum for all three EBV profile tests.

References

1. LIAISON® EBNA IgG (310520) Directions for Use, 10/2012, DiaSorin, Inc, Stillwater, MN 55082.
2. LIAISON® EBNA IgG Control Directions for Use, EBNA-G-us.fm, 200/007-863, C – 10/2012
3. EBV and CMV in Childhood Diseases, Sam Dunmire Presentation, Hogquist Lab, April 2011

Appendices

Refer to LIAISON® EBNA IgG (310520) Directions for Use for specific performance characteristics

Historical Record

Version	Written/Revised by:	Effective Date:	Summary of Revisions
1.	Linda Lichty	August 15, 2011	Initial Version
2.	Linda Lichty	August 22, 2011	Added statements for clarification of reporting, and QC handling
3.	Linda Lichty	April 22, 2013	Update package insert
4.	Linda Lichty	October 21, 2016	Revised Sample stability