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Changes: §4, §6;
Deletions: -

LIAISON® EBV IgM (REF 310500)

1. INTENDED USE

The LIAISON® EBV IgM assay uses chemiluminescence immunoassay (CLIA) technology for the quantitative determination of specific IgM antibodies to Epstein-Barr viral capsid antigens (VCA) in human serum or plasma samples. The test has to be performed on the LIAISON® Analyzer family*.

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 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, 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 percentage 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 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-1) 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-1, 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-1 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.

*(LIAISON®, LIAISON® XL, LIAISON® XS)

3. PRINCIPLE OF THE PROCEDURE

The method for quantitative determination of specific IgM to Epstein-Barr viral capsid antigens (VCA) is an indirect chemiluminescence immunoassay (CLIA). p18 synthetic peptide is the major component used for coating magnetic particles (solid phase) and a mouse monoclonal antibody is linked to an isoluminol derivative (isoluminol-antibody conjugate). During the first incubation, VCA antibodies present in calibrators, samples or controls bind to the solid phase. During the second incubation, the antibody conjugate reacts with VCA IgM already bound to the solid phase. After each incubation, the unbound material is removed with a wash cycle.

Subsequently, the starter reagents are added and a flash chemiluminescence reaction is thus 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 VCA IgM concentration present in calibrators, samples or controls. Buffer A contains goat IgG to human IgG as an absorbent reagent to curb interference from human IgG specific to VCA or from rheumatoid factor.

4. MATERIALS PROVIDED

Reagent integral

| | | |
|-----------------------------|----------------|---|
| Magnetic particles (2.3 mL) | [SORB] | Magnetic particles coated with Epstein-Barr viral capsid antigens, BSA, phosphate buffer, < 0.1% sodium azide. |
| Calibrator 1 (0.55 mL) | [CAL1] | Human serum/plasma containing low EBV IgM levels, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert yellow dye. The calibrator concentrations (U/mL) are referenced to an in-house antibody preparation. |
| Calibrator 2 (0.55 mL) | [CAL2] | Human serum/plasma containing high EBV IgM levels, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. The calibrator concentrations (U/mL) are referenced to an in-house antibody preparation. |
| Buffer A (25 mL) | [BUF A] | Goat IgG to human IgG (absorbent reagent), goat serum, BSA, phosphate buffer, 0.2% ProClin™ 300, an inert blue dye. |
| Conjugate (23 mL) | [CONJ] | Mouse monoclonal antibodies to human IgM conjugated to an isoluminol derivative, non-specific IgG (mouse polyclonal), BSA, phosphate buffer, 0.2% ProClin™ 300, preservatives. |
| Number of tests | | 100 |

All reagents are supplied ready to use. The order of reagents reflects the layout of containers in the reagent integral.

Materials required but not provided (system related)

| LIAISON® XL Analyzer | LIAISON® Analyzer |
|--|--|
| LIAISON® XL Cuvettes ([REF] X0016). | LIAISON® Module ([REF] 319130). |
| LIAISON® XL Disposable Tips ([REF] X0015) or LIAISON® Disposable Tips ([REF] X0055). | – |
| – | – |
| LIAISON® XL Starter Kit ([REF] 319200) or LIAISON® EASY Starter Kit ([REF] 319300). | LIAISON® Starter Kit ([REF] 319102) or LIAISON® XL Starter Kit ([REF] 319200) or LIAISON® EASY Starter Kit ([REF] 319300). |
| – | LIAISON® Light Check 12 ([REF] 319150). |
| LIAISON® Wash/System Liquid ([REF] 319100). | LIAISON® Wash/System Liquid ([REF] 319100). |
| LIAISON® XL Waste Bags ([REF] X0025). | LIAISON® Waste Bags ([REF] 450003). |
| LIAISON® XL Cleaning Tool ([REF] 310995) or LIAISON® EASY Cleaning Tool ([REF] 310996). | LIAISON® Cleaning Kit ([REF] 310990). |
| | – |

| LIAISON® XS Analyzer |
|---|
| LIAISON® Cuvettes on Tray ([REF] X0053). |
| LIAISON® Disposable Tips ([REF] X0055). |
| LIAISON® EASY Starter Kit ([REF] 319300). |
| LIAISON® EASY Wash Buffer ([REF] 319301). |
| LIAISON® EASY System Liquid ([REF] 319302). |
| LIAISON® EASY Waste ([REF] X0054). |
| LIAISON® EASY Cleaning Tool ([REF] 310996). |

Additionally required materials

LIAISON® EBV IgM controls (negative and positive) (**[REF]** 310501).

5. WARNINGS AND PRECAUTIONS

For *in vitro* diagnostic use.

All serum and plasma units used to produce the components provided in this kit have been tested for the presence of HBsAg, anti-HCV, anti-HIV-1, anti-HIV-2 and found to be non-reactive. As, however, no test method can offer absolute assurance that pathogens are absent, all specimens of human origin should be considered potentially infectious and handled with care.

6. SAFETY PRECAUTIONS

Do not eat, drink, smoke or apply cosmetics in the assay laboratory.

Do not pipette by mouth.

Avoid direct contact with potentially infected material by wearing laboratory clothing, protective goggles, and disposable gloves. Wash hands thoroughly at the end of each assay.

Avoid splashing or forming an aerosol. All drops of biological reagent must be removed with a sodium hypochlorite solution with 0.5% active chlorine, and the means used must be treated as infected waste.

All samples and reagents containing biological materials used for the assay must be considered as potentially able to transmit infectious agents. The waste must be handled with care and disposed of in compliance with the laboratory guidelines and the statutory provisions in force in each Country. Any materials for reuse must be appropriately sterilized in compliance with the local laws and guidelines. Check the effectiveness of the sterilization/decontamination cycle.

Do not use kits or components beyond the expiration date given on the label.

Pursuant to EC Regulation 1272/2008 (CLP) hazardous reagents are classified and labelled as follow:

| | |
|---|---|
| REAGENTS: | CAL1, CAL2, BUFA, CONJ |
| CLASSIFICATION | Skin sens. 1A H317 Aquatic chronic 3 H412 |
| SIGNAL WORD: | Warning |
| SYMBOLS / PICTOGRAMS: |  GHS07 Exclamation mark |
| HAZARD STATEMENTS: | H317 May cause an allergic skin reaction. H412 Harmful to aquatic life with long lasting effects. |
| PRECAUTIONARY STATEMENTS: | P261 Avoid breathing dust/fume/gas/mist/vapours/spray. P280 Wear protective gloves/protective clothing/eye protection/face protection. P273 Avoid release to the environment. P362 Take off contaminated clothing and wash before reuse. |
| CONTAINS: (only substances prescribed pursuant to Article 18 of EC Regulation 1272/2008). | reaction mass of: 5-chloro-2-methyl-4-isothiazolin-3-one [EC no. 247-500-7] and 2-methyl-2H -isothiazol-3-one [EC no. 220-239-6] (3:1) (ProClin™ 300). |

Pursuant to EC Regulation 1272/2008 (CLP), SORB is labelled as EUH210 safety data sheets available on request. For additional information see Safety Data Sheets available on www.diasorin.com.

7. PREPARATION OF REAGENT INTEGRAL

Please note the following important reagent handling precautions:

Resuspension of magnetic particles

Magnetic particles must be completely resuspended before the integral is placed on the instrument. Follow the steps below to ensure complete suspension:

Before the seal is removed, rotate the small wheel at the magnetic particle compartment until the colour 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). Visually check the bottom of the magnetic particle vial to confirm that all settled magnetic particles have resuspended. Carefully wipe the surface of each septum to remove residual liquid.

Repeat as necessary until the magnetic particles are completely resuspended.

Foaming of reagents

In order to ensure optimal performance of the integral, foaming of reagents should be avoided. Adhere to the recommendation below to prevent this occurrence:

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.

Loading of integral into the reagent area

LIAISON® Analyzer

- Place the integral into the reagent area of the analyzer with the bar code label facing left and let it stand for 30 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

LIAISON® XL and LIAISON® XS analyzers

- LIAISON® XL Analyzer and LIAISON® XS Analyzer are equipped with a built-in solid-state magnetic device which aids in the dispersal of microparticles prior to placement of a reagent integral into the reagent area of the analyzer. Refer to the analyzer operator's manual for details.
 - a. Insert the reagent integral into the dedicated slot.
 - b. Allow the reagent integral to remain in the solid-state magnetic device for at least 30 seconds (up to several minutes). Repeat as necessary.
- Place the integral into the reagent area of the analyzer with the label facing left and let it stand for 15 minutes before using. The analyzer automatically stirs and completely resuspends the magnetic particles.
- Follow the analyzer operator's manual to load the specimens and start the run.

8. STORAGE AND STABILITY OF REAGENT INTEGRAL

Upon receipt, the reagent integral must be stored in an upright position to facilitate resuspension of magnetic particles. When the reagent integral is stored sealed and kept upright, the reagents are stable at 2-8°C up to the expiry date. Do not freeze. The reagent integral should not be used past the expiry date indicated on the kit and reagent integral labels. After removing the seals, the reagent integral is stable for eight weeks refrigerated either at 2-8°C or on board the instrument.

9. SPECIMEN COLLECTION AND PREPARATION

Either human serum or plasma may be used. The anticoagulants citrate, EDTA and heparin have been tested and may be used with this assay. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Samples having particulate matter, turbidity, lipaemia, or erythrocyte debris may require clarification by filtration or centrifugation before testing. Grossly haemolyzed or lipaemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination should not be tested. Check for and remove air bubbles before assaying. If the assay is performed within seven days of sample collection, the samples may be kept at 2-8°C; otherwise they should be aliquoted and stored deep-frozen (-20°C or below). If samples are stored frozen, mix thawed samples well before testing. Four samples with different reactivity were stored for seven days at 2-8°C and underwent five freeze-thaw cycles. The results showed no significant differences. The minimum volume required is 170 µL specimen (20 µL specimen + 150 µL dead volume).

10. 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.

Recalibration in triplicate is mandatory whenever at least one of the following conditions occurs:

- A new lot of reagent integral or of Starter Kit is used.
- The previous calibration was performed more than four weeks before.
- **LIAISON® and LIAISON® XL analyzers:** The analyzer has been serviced.
- **LIAISON® XS Analyzer:** After a technical intervention, only if required by the service procedure, as communicated by local DiaSorin technical support or representative.
- Control values lie outside the expected ranges.

LIAISON® Analyzer: Calibrator values are stored in the bar codes on the integral label.

LIAISON® XL Analyzer: Calibrator values are stored in the reagent integral Radio Frequency Identification transponder (RFID Tag).

LIAISON® XS Analyzer: Calibrator values are stored in the reagent integral Radio Frequency Identification Transponder (RFID Tag).

11. ASSAY PROCEDURE

Strict adherence to the analyzer operator's manual ensures proper assay performance.

LIAISON® Analyzer. Each test parameter is identified via the bar codes on the reagent integral label. In the event that the barcode label cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

LIAISON® XL and LIAISON® XS analyzers. Each test parameter is identified via information encoded in the reagent integral Radio Frequency Identification transponder (RFID Tag). In the event that the RFID Tag cannot be read by the analyzer, the integral cannot be used. Do not discard the reagent integral; contact your local DiaSorin technical support for instruction.

The analyzer operations are as follows:

1. Dilute samples and controls with Buffer A.
2. Dispense magnetic particles and Buffer A.
3. Dispense calibrators, controls or specimens into the reaction module.
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.

Warning - Maintenance with the LIAISON® XL Cleaning Tool ([REF](#) 310995) or LIAISON® EASY Cleaning Tool ([REF](#) 310996) must be performed (refer to pertinent instruction for use for details).

12. QUALITY CONTROL

LIAISON® controls should be run in singlicate to monitor the assay performance. Quality control must be performed by running LIAISON® EBV IgM controls

- (a) at least once per day of use,
- (b) whenever a new reagent integral is used,
- (c) whenever the kit is calibrated,
- (d) whenever a new lot of Starter Reagents is used,
- (e) to assess adequacy of performance of the open integral in agreement with guidelines or requirements of local regulations or accredited organizations.

Control values must lie within the expected ranges: whenever one or both controls lie outside the expected ranges, calibration should be repeated and controls retested. If control values obtained after successful calibration lie repeatedly outside the predefined ranges, the test should be repeated using an unopened control vial. If control values lie outside the expected ranges, patient results must not be reported.

The performance of other controls should be evaluated for compatibility with this assay before they are used. Appropriate value ranges should then be established for quality control materials used.

13. INTERPRETATION OF RESULTS

The analyzer automatically calculates EBV IgM antibody concentrations expressed as U/mL and grades the results. For details, refer to the analyzer operator's manual.

Calibrators and controls may give different RLU or dose results on LIAISON®, LIAISON® XL and LIAISON® XS, but patient results are equivalent.

Assay range. 10 to 160 U/mL EBV IgM.

Samples containing antibody levels above the assay range may be prediluted by the Dilute function of the instrument and retested (the recommended dilution factor is 1:10). The results will then be automatically multiplied by the dilution factor to obtain the antibody levels of the neat specimens. The specimen diluent excess available in the reagent integral allows up to 14 sample predilutions to be performed.

Sample results should be interpreted as follows:

Samples with EBV IgM concentrations below 20 U/mL should be graded *negative*.

Samples with EBV IgM concentrations ranging between 20 and 40 U/mL should be graded *equivocal*. Detection of additional EBV markers may provide useful information for clinical interpretation of results.

Samples with EBV IgM concentrations equal to or above 40 U/mL should be graded *positive*.

A positive result is indicative of recent infection. A negative result, however, does not always rule out acute infection. If exposure to Epstein-Barr virus is suspected despite a negative finding, a second sample should be collected no less than one or two weeks later and should be tested to look for a significant rise in EBV IgM or IgG levels, which is indicative of primary infection. An equivocal result is indicative either of primary EBV infection or of past infection with long-lasting EBV IgM. Serological data from detection of additional EBV markers may provide useful information for clinical interpretation of results. Specific IgM antibodies are usually detected in patients with recent primary infection and may be found in patients with reactivated infections. However, low titres of IgM to EBV may be found in patients with no detectable evidence of recent infection (e.g. active chronic EBV infection).

Test results are reported quantitatively as positive or negative for the presence of EBV IgM. However, diagnosis of infectious diseases should not be established on the basis of a single test result, but should be determined in conjunction with clinical findings and other diagnostic procedures as well as in association with medical judgement.

Parallel determination of specific VCA IgG, EBNA IgG and EBV IgM levels enables better discrimination between different phases of EBV infection. Whenever multiple LIAISON® EBV tests are performed, a different cut-off may be used for more correct interpretation of EBNA IgG and EBV IgM results. The following interpretation of results is recommended.

| EBV IgM result | VCA IgG result | EBNA IgG result | Interpretation |
|----------------|----------------|-----------------|--|
| < 20 U/mL | < 20 U/mL | < 20 U/mL | EBV negative. |
| ≥ 20 U/mL | < 20 U/mL | < 20 U/mL | Suspected primary EBV infection (early phase). |
| ≥ 20 U/mL | ≥ 20 U/mL | < 20 U/mL | Primary EBV infection (acute phase). |
| ≥ 40 U/mL | ≥ 20 U/mL | ≥ 20 U/mL | Primary EBV infection (transient phase). |
| < 40 U/mL | ≥ 20 U/mL | ≥ 20 U/mL | Past EBV infection or reactivation. |
| < 20 U/mL | ≥ 20 U/mL | ≥ 5 U/mL | Past EBV infection or reactivation. |
| < 20 U/mL | ≥ 20 U/mL | < 5 U/mL | Unresolved (VCA IgG positive only). |
| Other results | | | Unknown. |

14. LIMITATIONS OF THE PROCEDURE

The combined use of EBV serological markers and clinical data is recommended when the diagnosis of acute EBV infection is based on a single specimen. A single result should not be used for diagnosis.

Assay performance characteristics have not been established when any LIAISON® EBV test is used in conjunction with other manufacturers' assays for detection of specific EBV serological markers. Under these conditions, users are responsible for establishing their own performance characteristics.

A skillful technique and strict adherence to the instructions are necessary to obtain reliable results.

Bacterial contamination or heat inactivation of the specimens may affect the test results.

Integrals may not be exchanged between analyzer types (LIAISON®, LIAISON® XL and LIAISON® XS). Once an integral has been introduced to a particular analyzer type, it must always be used on that analyzer until it has been exhausted. Due to traceability issues resulting from the above statement, patient follow-ups may not be concluded between analyzer types. These must be accomplished on one particular analyzer type (either LIAISON®, LIAISON® XL or LIAISON® XS).

15. SPECIFIC PERFORMANCE CHARACTERISTICS

15.1. Analytical specificity

Analytical specificity may be defined as the ability of the assay to accurately detect specific analyte in the presence of potentially interfering factors in the sample matrix (e.g., anticoagulants, haemolysis, effects of sample treatment), or cross-reactive antibodies.

Interference. Controlled studies of potentially interfering substances or conditions showed that the assay performance was not affected by anticoagulants (sodium citrate, EDTA, heparin), haemolysis (up to 1000 mg/dL haemoglobin), lipaemia (up to 3000 mg/dL triglycerides), bilirubinaemia (up to 20 mg/dL bilirubin), or by freeze-thaw cycles of samples.

Cross-reactions. The cross-reactivity study for the LIAISON® EBV IgM assay was designed to evaluate potential interference from antibodies to other organisms (hCMV, parvovirus B19, *Toxoplasma gondii*, rubella virus, HBV, HAV, HSV, *Treponema pallidum*, VZV, measles virus, mumps virus, *Borrelia burgdorferi*, influenza viruses) as well as from other conditions that may result from atypical immune system activity (anti-nuclear autoantibodies, rheumatoid factor). Samples for these studies were pre-screened with another commercially available EBV IgM assay. If found negative for EBV IgM antibodies those specimens were used to study potential cross-reactivity. The presence of potential cross-reactants in the samples was detected using CE-marked assays.

| Condition | Number of expected negative samples | LIAISON® positive results |
|--|-------------------------------------|---------------------------|
| Parvovirus B19 IgM antibodies | 70 | 7 |
| Rubella virus IgM antibodies | 130 | 14 |
| <i>Toxoplasma gondii</i> IgM antibodies | 135 | 0 |
| HAV IgM antibodies | 66 | 1 |
| HBc IgM antibodies | 33 | 1 |
| hCMV IgM antibodies | 44 | 2 |
| HSV-1/2 IgM antibodies | 15 | 0 |
| Influenza viruses antibodies | 7 | 0 |
| <i>Borrelia burgdorferi</i> IgM antibodies | 9 | 1 |
| Mumps virus IgM antibodies | 10 | 0 |
| Measles virus IgM antibodies | 6 | 0 |
| VZV IgM antibodies | 28 | 1 |
| <i>Treponema pallidum</i> IgM antibodies | 3 | 0 |
| Anti-nuclear autoantibodies (ANA/ENA) | 29 | 0 |
| Rheumatoid factor (anti-Fc immunoglobulin) | 18 | 0 |
| Total | 603 | 27 |

Assay interference may occur due to the presence of patient's IgM to rubella virus or parvovirus B19 during early acute infection. Long-lasting IgM does not interfere in LIAISON® EBV IgM assay.

Reactivity may be related to cross-reactions with early IgM to other viruses or to reappearance of EBV IgM as a consequence of polyclonal activation induced by other agents that caused an infectious mononucleosis-like syndrome.

15.2. Precision with LIAISON® Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

| Repeatability | A | B | C | D | E |
|------------------------------|------|------|------|------|------|
| Number of determinations | 20 | 20 | 20 | 20 | 20 |
| Mean (U/mL) | 11.0 | 32.0 | 59.0 | 94.0 | 98.0 |
| Standard deviation | 0.6 | 1.7 | 4.4 | 5.5 | 7.7 |
| Coefficient of variation (%) | 5.7 | 5.4 | 7.4 | 5.8 | 7.9 |

| Reproducibility | A | F | B | C | G |
|------------------------------|------|------|------|------|------|
| Number of determinations | 10 | 10 | 10 | 10 | 10 |
| Mean (U/mL) | 8.0 | 14.0 | 31.0 | 54.0 | 81.0 |
| Standard deviation | 0.8 | 0.8 | 3.0 | 3.1 | 6.5 |
| Coefficient of variation (%) | 11.1 | 6.3 | 9.7 | 5.8 | 8.0 |

15.3. Precision with LIAISON® XL Analyzer

Different samples, containing different concentrations of specific analyte, were assayed to determine repeatability and reproducibility of the assay (i.e., within- and between-assay variability). The variability shown in the tables below did not result in sample misclassification.

Repeatability. Twenty replicates were performed in the same run to evaluate repeatability.

| Repeatability | 1 | 2 | 3 | 4 | 5 | 6 | 7 | Negative control | Positive control |
|------------------------------|------|------|------|------|------|------|-------|------------------|------------------|
| Number of determinations | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean (U/mL) | 15.8 | 25.3 | 38.0 | 41.4 | 61.0 | 71.5 | 107.4 | 7.90 | 94.2 |
| Standard deviation | 1.10 | 1.50 | 1.99 | 2.53 | 1.52 | 3.85 | 7.17 | 0.28 | 3.60 |
| Coefficient of variation (%) | 7.0 | 5.9 | 5.2 | 6.1 | 2.5 | 5.4 | 6.7 | 3.6 | 3.8 |
| Min. value | 13.3 | 22.6 | 33.6 | 34.1 | 57.6 | 61.4 | 96.6 | 7.30 | 87.6 |
| Max. value | 17.6 | 27.9 | 41.0 | 44.4 | 64.7 | 78.1 | 125 | 8.40 | 104 |

Reproducibility. Twenty replicates were performed in different days (one or two runs per day) to evaluate reproducibility.

| Reproducibility | 1 | 8 | 4 | 9 | 5 | 10 | 7 | Negative control | Positive control |
|------------------------------|------|------|------|------|------|------|-------|------------------|------------------|
| Number of determinations | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 | 20 |
| Mean (U/mL) | 16.0 | 22.7 | 36.4 | 37.4 | 60.0 | 69.3 | 97.7 | 8.60 | 99.9 |
| Standard deviation | 1.64 | 1.94 | 4.16 | 3.78 | 3.27 | 8.09 | 13.60 | 0.93 | 5.52 |
| Coefficient of variation (%) | 10.2 | 8.5 | 11.4 | 10.1 | 5.4 | 11.7 | 13.9 | 10.8 | 5.5 |
| Min. value | 13.3 | 19.2 | 29.7 | 31.7 | 54.3 | 57.6 | 66.5 | 7.80 | 93.4 |
| Max. value | 19.2 | 25.7 | 43.5 | 45.7 | 65.9 | 83.6 | 116 | 12.2 | 114 |

15.4. Precision with LIAISON® XS Analyzer

A five day precision study was conducted on three LIAISON® XS Analyzers to verify the precision with the LIAISON® EBV IgM Assay. The CLSI document EP15-A3 was consulted in the preparation of the testing protocol.

A coded panel comprised of 6 frozen samples was used for the study.

The samples could be prepared by pooling samples with similar title in order to represent negative, borderline and positive levels.

The LIAISON® Control EBV IgM set was also included in the five day study.

The coded panel was tested on three LIAISON® XS Analyzers, in six replicates in a single run per day, for 5 operative days.

The mean Index value, standard deviation, and coefficient of variation (%CV) of the results were computed for each of the tested specimens for each of the instruments and across instruments.

Repeatability. Ninety replicates were performed in the same test to evaluate repeatability. 6 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

| Repeatability | 11 | 12 | 13 | 14 | 15 | 16 | Negative control* | Positive control |
|------------------------------|------|------|------|------|------|------|-------------------|------------------|
| Number of determinations | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 |
| Mean (U/mL) | 17.8 | 28.3 | 63.4 | 105 | 89.3 | 118 | 2723 | 76.0 |
| Standard deviation | 0.79 | 1.02 | 2.37 | 3.41 | 2.37 | 5.35 | 82.6 | 1.88 |
| Coefficient of variation (%) | 4.4 | 3.6 | 3.7 | 3.2 | 2.7 | 4.5 | 3.0 | 2.5 |
| Min. value | 14.1 | 25.7 | 56.7 | 92.0 | 81.5 | 102 | 2479 | 66.8 |
| Max. value | 20.1 | 32.0 | 70.6 | 115 | 97.7 | 135 | 3028 | 86.2 |

*Negative Control is expressed in RLU because out of the Assay Range.

Reproducibility. Ninety replicates were performed in different days (one run per day) to evaluate reproducibility. 6 serum samples containing different concentration of analyte and kit controls were assayed in 6 replicates per day, over 5 operating days, on 3 units and one reagent lot.

| Reproducibility | 11 | 12 | 13 | 14 | 15 | 16 | Negative control* | Positive control |
|------------------------------|------|------|------|------|------|------|-------------------|------------------|
| Number of determinations | 90 | 90 | 90 | 90 | 90 | 90 | 90 | 90 |
| Mean (U/mL) | 17.8 | 28.3 | 63.4 | 105 | 89.3 | 118 | 2723 | 76.0 |
| Standard deviation | 0.99 | 1.30 | 2.74 | 4.27 | 2.83 | 5.46 | 124 | 4.09 |
| Coefficient of variation (%) | 5.5 | 4.6 | 4.3 | 4.0 | 3.2 | 4.6 | 4.6 | 5.4 |
| Min. value | 14.1 | 25.7 | 56.7 | 92.0 | 81.5 | 102 | 2479 | 66.8 |
| Max. value | 20.1 | 32.0 | 70.6 | 115 | 97.7 | 135 | 3028 | 86.2 |

*Negative Control is expressed in RLU because out of the Assay Range.

15.5. Trueness

The assay trueness has been checked by the dilution test.

Dilution test. Four serum samples containing high EBV IgM concentrations were tested as such and after serially diluting with buffer A. EBV IgM concentrations measured versus concentrations expected were analyzed by linear regression. The correlation coefficients (r) ranged from 0.997 to 0.998.

| Dilution | Expected concentration, U/mL | Measured concentration, U/mL | % Recovery | Dilution | Expected concentration, U/mL | Measured concentration, U/mL | % Recovery |
|----------|------------------------------|------------------------------|------------|----------|------------------------------|------------------------------|------------|
| neat | – | 62.3 | – | neat | – | 149.2 | – |
| 1:2 | 31.1 | 32.6 | 104.7 | 1:2 | 74.6 | 75.7 | 101.5 |
| 1:4 | 15.6 | 13.5 | 86.8 | 1:4 | 37.3 | 41.5 | 111.3 |
| 1:8 | 7.8 | 7.4 | 95.1 | 1:8 | 18.6 | 20.9 | 112.4 |
| 1:16 | 3.9 | 3.5 | 90.0 | 1:16 | 9.3 | 8.2 | 88.2 |
| neat | – | 91.3 | – | neat | – | 150.0 | – |
| 1:2 | 45.6 | 50.5 | 110.7 | 1:2 | 75.0 | 78.0 | 104.0 |
| 1:4 | 22.8 | 24.6 | 107.9 | 1:4 | 37.5 | 40.8 | 108.8 |
| 1:8 | 11.4 | 11.3 | 99.1 | 1:8 | 18.8 | 20.2 | 107.8 |
| 1:16 | 5.7 | 5.3 | 93.0 | 1:16 | 9.4 | 8.5 | 90.7 |
| 1:32 | 2.9 | 3.0 | 105.3 | 1:32 | 4.7 | 4.2 | 89.6 |
| | | | | 1:64 | 2.3 | 2.3 | 98.2 |

15.6. High-dose saturation effect

Whenever samples containing extremely high antibody concentrations are tested, the saturation effect can mimic concentrations lower than real. However, a well-optimized two-step method excludes grossly underestimated results, because the analytical signals remain consistently high (saturation curve).

Analysis of saturation effect was evaluated by testing three high-titred samples positive for EBV IgM. All samples resulted in concentration values above the assay range that would be expected with high-titred sera, indicating no sample misclassification.

15.7. Diagnostic specificity and sensitivity

Diagnostic specificity and sensitivity were assessed by testing 2336 specimens from different selected populations (subjects never infected by EBV, apparently healthy subjects, subjects affected by autoimmune diseases, patients affected by other infectious diseases with similar symptomatology, patients affected by primary EBV infection, subjects with past EBV infection, patients with suspected chronic EBV infection, patients affected by reactivated EBV infection). The specimens were tested by several comparison methods and consensus between them as well as the available clinical and serological data were applied to define the expected results. 392 specimens were unresolved either by the method under test or by the reference methods and therefore were not included in the data analysis.

13 positive and 1654 negative results were observed in the expected negative population studied - diagnostic specificity: 99.22% (95% confidence interval: 98.67-99.58%).

Six negative and 271 positive results were observed in the expected positive population studied - diagnostic sensitivity: 97.83% (95% confidence interval: 95.35-99.20%).

15.8. EBV reactivity pattern

2343 samples from different selected populations were tested during performance evaluation studies: subjects never infected by EBV, apparently healthy adult subjects, subjects affected by autoimmune diseases, patients affected by other infectious diseases, patients affected by primary EBV infection, subjects with past EBV infection, patients with suspected chronic EBV infection, patients affected by reactivated EBV infection.

The three LIAISON® EBV test results were combined to identify the phase of EBV infection from a serological point of view and to evaluate the ability of multiple LIAISON® EBV tests to correctly classify the samples.

The serological diagnosis arising from multiple LIAISON® EBV tests was compared with the results obtained from the comparison tests for the most representative categories of subjects, such as EBV-negative subjects, patients affected by primary EBV infection and subjects with past EBV infection.

Out of 210 expected EBV-negative samples, 181 samples showed a negative LIAISON® EBV pattern. Concordance with the comparison pattern was therefore 86.20% (95% confidence interval: 80.77%-90.56%).

Out of 282 expected primary EBV infection samples, 255 samples showed a pattern of primary EBV infection by LIAISON®. Concordance with the comparison pattern was therefore 90.43% (95% confidence interval: 86.37%-93.59%).

Out of 1616 expected past EBV infection samples, 1479 samples showed a pattern of past EBV infection by LIAISON®. Concordance with the comparison pattern was therefore 91.52% (95% confidence interval: 90.05%-92.84%).

Interpretation of results obtained with multiple LIAISON® EBV tests can be directly performed by the instrument through Dia-Link software.

| LIAISON® EBV pattern | EBV IgM | VCA IgG | EBNA IgG | No. of subjects | Percentage |
|------------------------|----------|----------|----------|-----------------|------------|
| EBV-negative subjects | negative | negative | negative | 202 | 8.6% |
| Primary EBV infection | | | | | |
| . early phase | positive | negative | negative | 113 | 4.8% |
| . acute phase | positive | positive | negative | 221 | 9.4% |
| . transient phase | positive | positive | positive | 132 | 5.6% |
| Past EBV infection | negative | positive | positive | 1594 | 68.1% |
| Unresolved EBV pattern | negative | positive | negative | 32 | 1.4% |
| Unknown EBV pattern | negative | negative | positive | 49 | 2.1% |
| TOTAL | | | | 2343 | 100% |

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