ReQuest® EBV EA-D IgG

REF 01-490 96-Test Set

IVD

Intended Use

The ReQuest® EBV EA-D IgG test is for the qualitative detection of human IgG antibodies to Epstein-Barr virus early antigen diffuse (EA-D) in human serum by enzyme immunoassay. This assay uses a 28 kd *E. coli* expressed recombinant Epstein-Barr virus early antigen. When performed in conjunction with other EBV serological tests, this assay can be used as an aid in the laboratory diagnosis of EBV infectious mononucleosis in patients with signs and symptoms of EBV infectious mononucleosis. For In Vitro Diagnostic Use Only. Assay performance characteristics have not been established for neonatal, immunocompromised populations, cord blood, infants or pre-transplant patients. Assay performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, and others.

Summary and Explanation of Test

Epstein-Barr virus (EBV) is the etiological agent of infectious mononucleosis (IM). The designation of infectious mononucleosis classically refers to an Epstein-Barr virus-induced illness in young adults characterized by reactive blood smears, exudative pharyngitis, prominent cervical lymphadenopathy and serologically detectable heterophil antibodies. These clinical manifestations can also be caused by a number of other pathogenic agents including cytomegalovirus, Toxoplasma gondii, rubella virus, hepatitis virus, human immunodeficiency virus (HIV), and uncommonly by drugs such as Halothane, Hydantoin, Dapasone and Azulfidine (2, 3, 4).

Diagnosis of acute EBV IM is generally confirmed by a positive heterophil antibody test. The severity of the disease however, is not indicated by the relative titer of heterophil antibodies (5). In addition, difficulties in diagnosis arise when the heterophil antibody test is negative, or when the clinical manifestations are atypical or unusually severe.
Heterophil-negative IM occurs in 10 to 20 percent of adults, and in an even greater percentage of children (6, 7). IM diagnosis in these individuals may be confirmed by the detection and identification of antibodies to specific EB antigens which include: viral capsid antigen (VCA), early antigens, diffuse and restricted (EA-D and EA-R), and Epstein-Barr nuclear antigen (EBNA).

Antibodies to EBV-specific early antigens (EA), designated diffuse (Anti EA-D) or restrictive (Anti EA-R), depending on distinctive patterns of immunofluorescence and reaction to cell-line fixatives, become detectable in the acute phase of EBV-IM in most (80 to 90%) patients. With recovery and establishment of a latent, persistent EBV carrier state in B lymphocytes, anti-EA titers decline but may take 2 to 3 years, even in apparently healthy individuals, to reach baseline levels (1:80 or lower). Anti-EA (D) titers are not necessary for acute phase diagnosis.

The EBV EA-D EIA test is an ELISA test which utilizes a microwell format. Test results are obtained after one and one-half hours incubation time. They are objective and normalized as Index values, permitting uniformity of reporting.

Principle of the Test

Diluted samples are incubated in antigen-coated wells. Antibodies to EA-D antigen (if present) are bound to the EA-D antigen and immobilized on the wells. Residual sample is eliminated by washing and draining, and conjugate (enzyme labeled antibodies to human IgG) is added and incubated. If antibodies to EA-D are present, the conjugate will be immobilized on the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end product which is read photometrically.

Materials Provided

Coated Wells	Coated with 28 kd recombinant antigen of Epstein Barr Virus Early Antigen (diffuse) expressed in *E. coli.*
Well Support	One
Diluent*	25 ml (pink color). Contains phosphate buffered saline with 6% BSA as a protein stabilizer, pH 7.4 ± 0.2.
Calibrator*	0.3 ml. Human serum. Strongly reactive for antibodies to EBV EA-D. The Calibrator index value is printed on the vial label.
Positive Control*	0.3 ml. Human serum. Reactive for antibodies to EBV EA-D. The Positive Control index range is printed on the vial label.
Negative Control*	0.3 ml. Human serum. Nonreactive for antibodies to EBV EA-D.
Conjugate	12 ml (green color). Goat anti-human labeled with Alkaline phosphatase (calf).
Substrate	12 ml. P-nitrophenyl phosphate in 0.1 M bicarbonate buffer, pH 9.6 ± 0.2.
Wash Concentrate*	30 ml. Tris-buffered saline with 0.75 % Tween 20.Prepare Wash Solution by adding the contents of the Wash Concentrate bottle to 1 liter of distilled or deionized water.
Stop Reagent	12 ml. 0.5M Sodium Phosphate Tribasic, pH > 12.0.

* Contains 0.09% sodium azide.

Store these reagents according to the instructions on the bottle labels. Do not allow them to contact the skin or eyes. If contact occurs, wash with copious amounts of water.

Materials Required But Not Provided

1. Wash bottle
2. Pipettors for dispensing 4, 100 and 200 µl
3. Timer

CE

4. 1 or 2 liter container for Wash Solution
5. Distilled or deionized water
6. Dilution tubes or microwells
7. Microwell reader capable of reading absorbance at 405 nm.

Warnings and Precautions

1. For in vitro diagnostic use.
2. The presence of anti-EA D in a given specimen determined with assays from different manufacturers can vary due to differences in assay methods and reagent specificity.
3. The calibrators and controls have been found to be negative for HIV, hepatitis B surface antigen and HCV antibodies by FDA-approved methods. However, because no method can offer complete assurance that HIV, hepatitis B virus, HCV or other infectious agents are absent, these materials should be handled at the Biosafety Level 2 as recommended for any potentially infectious serum or blood specimen in the Centers for Disease Control/National Institutes of Health Manual "Biosafety in Microbiological and Biomedical Laboratories",5th Edition, 2007, and CLSI / NCCLS Approved Guideline, "Protection of Laboratory Workers from Occupationally Acquired Infections, 3rd Edition, 2007.
4. 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.
5. Avoid direct contact with all potentially infectious materials by using protective clothing such as lab coats, protective glasses and disposable gloves. Wash hands thoroughly at the end of each assay.
6. Avoid splashing or forming an aerosol. Any sample or reagent spills should be washed with a 5% sodium hypochlorite solution and disposed of as though potentially infectious.
7. Do not pipet solutions by mouth.
8. Some test reagents contain sodium azide as preservatives. Because sodium azide may form explosive lead or copper azide in plumbing, it is recommended to flush drains with large volumes of water when disposing of solutions containing sodium azide to minimize the build-up of metal-azide compounds.
9. Do not interchange reagents from different reagent lots.
10. Do not use reagents beyond their stated expiration date.
11. Incubation times recommended in the Test Procedure section must be adhered to.
12. Unused Coated Wells should be kept in their reseal able bag with desiccant, and stored in the refrigerator.

Specimen Collection and Storage

This assay can only test human serum samples. Blood should be collected aseptically by venipuncture, allowed to clot, and the serum separated from the clot as soon as possible. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested. Do not heat inactivate serum samples before testing. If specimens are not tested within 8 hours, they should be stored at 2 to 8^0C.for up to 48 hours. Beyond 48 hours specimens should be dispensed in aliquots and stored at -20^0C or below. Multiple freeze-thaw cycles should be avoided. Self-defrosting freezers are not recommended for sample storage.

Test Procedure

Allow all reagents and patient samples to reach room temperature before use. Return them promptly to refrigerator after use.

1. Prepare 1:51 dilutions of test samples, Calibrator, Positive and Negative Controls, in the test set Diluent. For example: add 4 µl of sample to 200 µl of Diluent in a dilution well or tube, and mix well.
2. Place an appropriate number of Coated Wells in the Well Support.
3. Transfer 100 µl of the diluted Calibrator, Controls and patient samples to the wells.

Note: Include one well which contains 100 µl of Diluent only. This will serve as the reagent blank and will be ultimately used to zero the photometer before reading the test results.

4. Incubate the wells at room temperature (20 to 25° C.) for 30 minutes.
5. Drain wells thoroughly by aspiration, or by inverting on a paper towel. Wash wells four times by filling the wells with at least 250 - 300 µL Wash Solution. Drain wells thoroughly after each wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
6. Place 100 µl of Conjugate into each well.
7. Incubate the wells at room temperature for 30 minutes.
8. Drain wells thoroughly. Wash wells four times with at least 250 µL Wash Solution/well/wash. Do not allow the wells to soak between washes. Drain thoroughly after the last wash.
9. Place 100 µl of Substrate into each well.
10. Incubate at room temperature for 30 minutes.
11. Place 100 µl of Stop Reagent into each well.
12. Read and record the absorbance of the contents of each well at 405 nm against the reagent blank.

Note: Adjust the photometer to zero absorbance at 405 nm against the reagent blank. Readings should be made within 2 hours after the reactions have been stopped at room temperature.

Calculation of Results

Determine the Index value for each test sample (or Control) using the following formula:

$$\frac{\text{Calibrator Index}}{\text{Calibrator Absorbance}} \times \text{Test Sample Absorbance} = \text{Test Sample Index}$$

Note: If the Calibrator is run in duplicate, use the average absorbance value to calculate results. The Calibrator index value is printed on the vial label.

Quality Control

1. The Calibrator, Positive and Negative Controls must be included in each test run.
2. The absorbance value of the Reagent Blank should be less than 0.35.
3. The Negative Control must have an index value less than 0.9.
4. The Positive Control must have an index value within the range printed on the label.

If any of these criteria are not met, the test is invalid and should be repeated.

5. The Positive and Negative Controls are intended to monitor for substantial reagent failure. The Positive Control will not ensure precision at the assay cutoff. Users may wish to establish an in-house control, having a quantitative value determined by replicate testing, at or near the cutoff of the assay, to monitor the precision of the assay cutoff. Additional controls may be tested according to guidelines or requirements of local, state and/or federal regulations or accrediting organizations. For guidance on appropriate quality control practices, please refer to CLSI Approved Guideline, C24-A3, "Statistical Quality Control for Quantitative Measurement Procedures; Principles and Definitions", 2006.

Interpretation of Results

Index	Interpretation	
< 0.9	Negative	No detectable antibody to EBV Early Antigen D
≥ 0.9 to < 1.1	Equivocal	Sample should be retested, please see No.1, below.
≥ 1.1	Positive	Detectable antibody to EBV Early Antigen D present.

1. Samples that remain equivocal after retesting should be tested by another serological method such as the immunofluorescence assay (IFA). If the sample remains equivocal after repeat testing an additional specimen should be acquired.

2. To determine the assay cut-off value, ninety-one (91) serum specimens that were previously shown to be negative by another legally marketed enzyme immunoassay were tested by the SeraQuest EBV EA-D IgG test. The mean and standard deviation of the index values obtained for the 91 negative specimens were 0.5 and 0.3, respectively. Based on these results the positive cut-off value for the test was set at 1.1, the mean plus two standard deviations. This cut-off was then validated in tests of prospectively obtained patient sera in parallel with another legally marketed commercial test.

3. In cases of heterophil antibody negative specimens, four distinct EBV antibodies are used to provide a comprehensive assessment of EBV infection. These antibodies are IgM antibodies directed against EBV viral capsid antigen, IgG antibodies against viral capsid antigen, IgG antibody to EBV early antigen and IgG antibody to EBV nuclear antigen (EBNA). An accurate interpretation of possible EBV infection should be based on an assessment of all of these antibodies, and should not be based on a single test result.

4. Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported antibody level cannot be correlated to an end point titer.

Limitations

1. The results obtained with the EBV EA-D EIA test serve only as an aid to diagnosis and are not by themselves diagnostic. Test results should be evaluated in association with patient symptoms, clinical data, and other laboratory findings.
2. The timing of the appearance of antibodies to EA-D is subject to variations among individuals and serological assays.
3. Some sera drawn very early at the onset of symptoms may not contain detectable levels of EA-D antibody and may require the drawing of another test specimen 1-2 weeks later.
4. Testing should not be performed as a screening procedure on the general population. The positive predicative value depends on the likelihood of the Epstein-Barr virus being present. Testing should only be performed on patients with clinical symptoms of infectious mononucleosis or when exposure is suspected. This assay should be used in conjunction with other EBV serological assays as an aid in the clinical diagnosis of infectious mononucleosis.
5. This assay is not intended for viral isolation and/or identification.
6. The assay should be performed on serum samples only. The assay's performance characteristics have not been established for matrices other than serum.
7. Assay performance characteristics have not been established for immunocompromised or immunosuppressed patients.
8. Assay performance characteristics have not been established for cord blood, neonatal or infant specimens.
9. Assay performance has not been adequately established in a pediatric population.

10. Performance characteristics have not been established for the diagnosis of nasopharyngeal carcinoma, Burkitt's lymphoma, EBV-associated lymphadenopathies, other EBV-associated lymphomas, and other EBV-associated diseases besides EBV- related mononucleosis.
11. Infections such as cytomegalovirus, toxoplasmosis and hepatitis may cause symptoms similar to infectious mononucleosis and must be excluded before confirmation of diagnosis.
12. This assay is intended for qualitative determination only. The numeric value of the final result above the cutoff is not indicative of the amount of EBV EA-D antibody. Performance characteristics for paired samples have not been established.
13. Assay cross-reactivity due to circulating antibodies against Human Immunodeficiency Virus (HIV), Hepatitis A, Hepatitis B, Hepatitis C viruses, Rubella, and Toxoplasma gondii has not evaluated. The user is responsible for establishing cross-reactivity performance with these infectious agents.
14. The assays performance characteristics have not been established for visual result determination.

Expected Values

Nearly all individuals have been infected with EBV by the time they reach adulthood (8). Characteristically, IgG antibodies to EA-D appear relatively early during IM infections.

The SeraQuest EBV EA-D IgG assay was tested with a total of 477 prospectively collected specimens from subjects sent to the laboratories (Laboratory A, B and C) for EBV testing to evaluate the prevalence of IgG antibodies to EBV EA-D in these populations.. Of the 477 prospectively collected and prospectively tested specimens, patient demographic information were available for 153 samples that were sent to and tested at Laboratory C. This particular patient cohort was comprised of 63% females and 37% males. Their ages ranged from 3 to 88 years, with an average age of 37.9 years. All serum samples in this patient cohort originated in Florida, Georgia and Alabama. Expected values for the SeraQuest EBV EA-D IgG Kit are presented by age and gender in Table 1 for serum samples from this particular patient cohort (N=153).

Table 1: Results of Tests of 153 Prospectively Obtained Serum Specimens Using the SeraQuest EBV EA-D IgG Test Performed at Laboratory C

			SeraQuest EBV EA-D IgG Result			
		N	Negative	Equivocal	Positive	Prevalence of EBV EA-D IgG
Total		153	89	10	54	35.3%
Gender						
	Female	97	49	8	40	41.2%
	Male	56	40	2	14	25.0%
Age (years)						
	<10	10	8	0	2	20.0%
	10 - 19	31	19	3	9	29.0%
	20 - 29	19	8	6	5	26.3%
	30 - 39	20	12	0	8	40.0%
	40 - 49	21	10	4	7	33.3%
	50 - 59	27	14	1	12	44.4%
	60 - 69	15	9	1	5	33.3%
	≥ 70	10	4	0	6	60.0%

Performance Characteristics

Clinical Performance Comparison

Performance of the SeraQuest EBV EA-D IgG Test was evaluated against another FDA cleared EBV EA-D IgG ELISA test according to the EBV serological characterization of the specimens as determined by other EBV serological reagents. For purposes of classifying the EBV serological state, specimens were tested by reference EBV serology assays for EBV VCA IgG, EBV VCA IgM, and EBV EBNA-1 IgG. The EBV EA-D IgG result generated by the FDA cleared comparator EBV EA-D IgG ELISA test was not considered for purposes of characterizing the EBV serological state of the specimen. A total of 542 serum samples for which EBV serology tests were ordered was tested at 3 U.S. clinical testing sites. Of the 542 specimens, 477 were prospectively collected and prospectively tested specimens, and 65 were prospectively collected but retrospectively tested specimens to supplement the prospective study data. Of the 65 prospectively collected but retrospectively tested specimens, 50 were acute specimens and 15 were EBV seronegative specimens characterized by reference EBV serology assays for EBV VCA IgG, EBV VCA IgM, and EBV EBNA-1 IgG. Based upon the results of the three reference EBV serology tests, the specimens were categorized into one of four EBV serological state groups as indicated in Table 2 below.

Table 2: EBV serological state characterization

	Specimen Group				
EBV serological	Prospectively Collected and	Prospectively Collected but retrospectively	EBV VCA IgG	EBV VCA	EBV EBNA-1

state	Prospectively Tested	Tested		IgM	IgG
Acute	31	50			
			+	+	-
			-	+	-
EBV seronegative	60	15			
			-	-	-
Past Infection	311	0			
			+	-	+
Indeterminate	75	0			
			-	-	+
			+	-	-
			-	+	+
			+	+	+
Total	477	65			

+ reactive; - nonreactive;
Note: When a reference assay was equivocal, it was considered nonreactive (-).
The characterization by antibody response profile was not compared with clinical data regarding presence, absence or status of disease.

Using Table 2 as a guideline, testing results were analyzed by the SeraQuest EBV EA-D IgG Test and corresponding comparative EBV EA-D IgG ELISA test according to the EBV serological characterization based on EBV serology reference assays results. For the purpose of percent agreement calculations, SeraQuest EBV EA-D IgG test equivocal results were assigned to the opposite test result interpretation than that of the corresponding comparative test results. Likewise, the comparative test equivocal results were assigned to the opposite test result interpretation than that of the corresponding SeraQuest EBV EA-D IgG Test results.

Prospectively collected and prospectively tested 477 sample results from all three sites combined are summarized in Tables 3-4.

Table 3: SeraQuest EBV EA-D IgG Test vs. Comparator Assay: Comparison by EBV Serological Status Characterization

EBV Serological Classification	Comparator EBV EA-D IgG Interpretation									Total
	Positive			Equivocal			Negative			
	SeraQuest EBV EA-D IgG			SeraQuest EBV EA-D IgG			SeraQuest EBV EA-D IgG			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute Infection	12	0	1	2	0	1	3	1	11	31
EBV Seronegative	4	2	1	0	1	5	2	4	41	60
Past Infection	65	13	11	7	8	12	17	14	164	311
Indeterminate	16	3	5	0	3	4	6	2	36	75
Overall	97	18	18	9	12	22	28	21	252	477

Table 4: SeraQuest EBV EA-D IgG Test vs. Comparator Assay: Percent Agreement & Confidence Intervals by EBV Serological Status Characterization

EBV Serological Status	Positive Agreement		95% CI	Negative Agreement		95% CI
Acute Infection	12/14	85.7%	57.2-98.2	11/17	64.7%	38.3-85.8

EBV Seronegative	4/12	33.3%	9.9-65.1	41/47	87.2%	74.3-95.2
Past infection	65/101	64.4%	55.0-73.7	164/202	81.2%	75.8-86.6
Indeterminate	16/28	57.1%	37.2-75.5	36/44	81.8%	67.3-91.8
Overall	97/155	62.6%	55.0-70.2	252/310	81.3%	76.9-85.6

Prospectively collected but retrospectively tested 65 specimen results from Site A are summarized in Tables 5-6.

Table 5: SeraQuest EBV EA-D IgG Test vs. Comparator Assay: Comparison by EBV Serological Status Characterization

EBV Serological Classification	Comparator EBV EA-D IgG Interpretation									Total
	Positive			Equivocal			Negative			
	SeraQuest EBV EA-D IgG			SeraQuest EBV EA-D IgG			SeraQuest EBV EA-D IgG			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute Infection	32	0	0	1	1	1	2	3	10	50
EBV Seronegative	0	0	3	0	0	1	1	1	9	15
Overall	32	0	3	1	1	2	3	4	19	65

Table 6: SeraQuest EBV EA-D IgG Test vs. Comparator Assay: Percent Agreement & Confidence Intervals by EBV Serological Status Characterization

EBV Serological Status	Positive Agreement		95% CI	Negative Agreement		95% CI
Acute Infection	32/33	97.0%	84.2- 99.9	10/16	62.5%	35.4 – 84.8
EBV Seronegative	0/4	0%	0 – 60.2	9/11	81.8%	48.2 – 97.7
Overall	32/37	86.5%	71.2- 95.5	19/27	70.4%	49.8 – 86.2

In addition, test results generated by both the SeraQuest EBV EA-D IgG Test and the comparator EA-D IgG Assay relative to the actual EBV serological characterization of either acute infection, EBV seronegative or past infection, as determined by the reference EBV serology assays for EBV VCA IgG, EBV VCA IgM, and EBV EBNA-1 IgG, for the 477 prospectively collected and tested specimens and the 65 prospectively collected but retrospectively tested specimens, are presented in Tables 7-8.

Table 7: Agreements of the Comparator EBV EA-D IgG Test, and the SeraQuest EBV EA-D IgG Test, relative to the EBV Serological Classification, for the prospectively collected and tested specimens

	Prospectively Collected and Tested			
	Comparator EBV EA-D IgG Test	95% CI	SeraQuest EBV EA-D IgG Test	95% CI
Positive Agreement (Acute Infection)	13/31 41.9%	24.5-60.9	17/31 54.8%	36.0-72.7
Negative Agreement (EBV Seronegative)	47/60 78.3%	65.8-87.9	47/60 78.3%	65.8-87.9
Negative Agreement (Past Infection)	195/311 62.7%	57.3-68.1	187/311 60.1%	54.7-65.6

Table 8: Agreements of the Comparator EBV EA-D IgG Test, and the SeraQuest EBV EA-D IgG Test, relative to the EBV Serological Classification, for the prospectively collected and retrospectively tested specimens

	Prospectively Collected and Retrospectively Tested			
	Comparator EBV EA-D IgG Test	95% CI	SeraQuest EBV EA-D IgG Test	95% CI
Positive Agreement (Acute Infection)	32/50 64.0%	49.2-77.1	35/50 70.0%	55.4-82.1
Negative Agreement (No Infection)	11/15 73.3%	44.9-92.2	13/15 86.7%	59.5-98.3

<u>Cross-reactivity</u>

The cross-reactivity study was designed to determine if samples from various disease states and other potentially interfering factors interfere with test results when tested with the SeraQuest EBV EA-D IgG Test. Specimens that were positive for various infectious diseases, heterophilic antibodies,

autoimmune antibodies and antibodies against other EBV markers were tested with the SeraQuest EBV EA-D IgG Test. Samples for these studies were selected using commercially available devices. Results can be found in Table 9.

Table 9: Cross-Reactivity

Analytes/Condition	Number of samples	Positive or Equivocal SeraQuest EBV EA-D IgG Test Result
Cytomegalovirus IgG	7	0/7
Herpes simplex virus 1&2 IgG	7	0/7
Varicella zoster virus IgG	11	0/11
Anti-Nuclear Antigen antibodies	2	0/2
Cytoplasmatic antigen SS-A antibodies	4	0/4
Cytoplasmatic antigen SS-B antibodies	4	0/4
Extractable nuclear antigen Sm antibodies	4	0/4
Cardiolipin IgG	6	0/6
Rheumatoid Factor	2	0/2
EBV VCA IgG	152	0/152
EBV VCA IgM	2	0/2
EBV NA antibodies	115	0/115
Total	316	0/316

None of the 316 total specimens tested in the cross-reactivity studies returned positive or equivocal results in the SeraQuest EBV EA-D IgG Test.

Warning: Potential cross-reactivity of the SeraQuest EBV EA-D IgG Test with IgG antibodies to *Toxoplasma gondii*, Rubella virus, HIV, HAV, HBV, and HCV was not tested and determined. The user is responsible for establishing cross-reactivity performance with these infectious agents.

Potential Interfering Substances

The possible effects of icterus, hemolysis, hyperglycemia, hyperlipidemia and hyperproteinemia, on the results of the SeraQuest EBV EA-D IgG test, were examined. A sample panel consisted of one weak positive serum sample (close to the assay cut-off) and one negative sample was prepared. Each serum specimen was first tested without any of the additive. This served as the control representing the normal physiological concentration of each of the potential interfering substances. In addition, aliquots of each serum specimen were supplemented with 8 times the normal level of each potential interferent. These levels were selected to exceed the levels that could be present in disease state sera. The normal and the "enriched" serum specimens with bilirubin, hemoglobin, glucose, cholesterol, and gamma globulin were tested following the SeraQuest EBV EA-D IgG Instructions for Use. Results can be found in Table 10.

Table 10: SeraQuest EBV EA-D IgG Test Results with Potential Interfering Substances

ANALYTE	ANALYTE CONCENTRATION			
	NORMAL		ELEVATED	
	POS (+) SAMPLE INDEX	NEG (-) SAMPLE INDEX	POS (+) SAMPLE INDEX	NEG (-) SAMPLE INDEX
BILIRUBIN	0.5 - 1.4 mg/dL		12 mg/dL	
	1.5	0.4	1.5	0.3
HEMOGLOBIN	0 gm/dL		18 gm/dL	
	1.4	0.4	1.3	0.4
GLUCOSE	60 -100 mg/dL		800 mg/dL	
	1.5	0.4	1.5	0.4
CHOLESTEROL	115 - 340 mg/dL		2,720 mg/dL	
	1.4	0.4	1.4	0.6
GLOBULIN	2.3 - 3.5 gm/dL		28 gm/dL	
	1.8	0.4	2.3	0.4

No significant interference was observed in the presence of up to eight times the normal physiological concentration of each of the potential interfering substances tested with the SeraQuest EBV EA-D IgG Test. There were no false negative results for the weak positive specimen and no false positive results for the negative specimens that were encountered in the presence of each of the potential interfering substances.

Warning: While the limited amount of data presented in the study above may not demonstrate it, serum specimens with elevated levels of these interfering substances may generate erroneous results. Grossly hemolyzed, icteric or lipemic samples as well as samples containing particulate matter or exhibiting obvious microbial contamination are not recommended and should not be tested

Precision

A reproducibility panel of 6 members was prepared by the Quest International laboratory. One (1) of the six panel members was negative for EBV EA-D IgG. One (1) of the 6 panel members had levels of EBV EA-D IgG near the assay cut-off that was considered a high negative to equivocal sample. Four (4) of the six panel members were positive for EBV EA-D IgG. All panel members were prepared from patient samples. This panel was split into aliquots and tested at 3 different clinical sites. In addition, 1 SeraQuest human Anti-EA-D IgG positive serum control and 1 SeraQuest human Anti-EA-D IgG negative serum control were also tested. Each of the 6 panel members and the SeraQuest positive and negative controls were tested three times (x3) on each day in one run for 3 days at each of the 3 US testing sites (3 times x 3 days x 3 sites = 27 replicates per panel member and SeraQuest control). The data was analyzed for intra-assay, inter-assay and between-site reproducibility. The standard deviation (SD) and percent coefficient of variation (%CV) were also calculated. Results can be found in Table 11.

Table 11: Reproducibility (Values were calculated from the SeraQuest index values.)

Name of analyte Panel Members	Sample N	Mean Index	Intra-Assay		Inter-Assay		Between-Site		Total	
			SD	%CV	SD	%CV	SD	%CV	SD	%CV
SeraQuest Positive Serum Control	27	1.7	0.05	3.1	0.13	8.0	0.32	19.3	0.14	8.0
SeraQuest Negative Serum Control	27	0.4	0.01	4.1	0.04	9.1	0.07	21.0	0.03	8.4
High negative to equivocal (Near C.O.)	27	0.7	0.04	5.7	0.07	9.5	0.10	14.2	0.03	4.2
Negative	27	0.2	0.05	18.2	0.06	23.1	0.12	46.7	0.04	14.0
Positive 1	27	1.7	0.08	4.2	0.09	5.8	0.40	23.8	0.19	10.5
Positive 2	27	1.3	0.07	5.2	0.07	5.9	0.26	19.5	0.11	7.9
Positive 3	27	1.3	0.04	3.0	0.08	6.6	0.26	20.5	0.12	8.9
Positive 4	27	1.7	0.09	4.7	0.24	14.1	0.40	24.1	0.16	9.2

References

1. Henle, W. and G. Henle.1981. Clinical spectrum of Epstein-Barr virus infection. In: *The Human Herpesviruses; An Interdisciplinary Perspective.* Nahmias, Dowdle and Schinazi,ed. Elsevier: New York.
2. Evans, A.S., IM and related syndromes.1978. Am. J. Med. Sci.; 276: 325-339.
3. Penman, H.G. 1969. The problem of seronegative IM. In: *Infectious Mononucleosis.* Carter, R.L. and H.G.Penman, eds. Oxford, Blackwell Scientific Publications, 201-224.
4. Horwitz, C.C., W. Henle, G. Henle, et al. 1977. Heterophil negative IM and mononucleosis-like illness. Laboratory confirmation of 43 cases. Am. J. Med. 63: 947-957.
5. Sumaya, C.V. 1986. Lab. Manag., 24: 23-28.
6. Lenette, E.T. 1987. Epstein-Barr Virus, Man. of Clin. Micro. 4th edition, Wash. D.C. Am. Soc. Micro., 728-732.
7. Henle, W., G.E. Henle and C.A. Horwitz. 1974. Hum. Pathol., 5: 551-565.
8. Hearth, C. W., A.L. Broasky and A.L. Potolsky, 1972, Am. J. Epidemiol., 95:46.

Manufacturer:
Quest International, Inc.
8127 NW 29th Street
Miami, FL 33122
USA

EMERGO EUROPE
Prinsessegracht 20
2514 AP The Hague
The Netherlands

L-01-490-9
2017-06

CE

Symbols Glossary

Symbol	Standard Title and Number	Title of Symbol	Symbol reference #	Explanatory Text
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Manufacturer	5.1.1	Indicates the medical device manufacturer, as defined in EU Directives 90/385/EEC, 93/42/EEC and 98/79/EC.
EC REP	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Authorized representative in the European Community	5.1.2	Indicates the Authorized representative in the European Community.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Use-by-date	5.1.4	Indicates the date after which the medical device is not to be used.
LOT	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Batch code	5.1.5	Indicates the manufacturer's batch code so that the batch or lot can be identified.
REF	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Catalog number	5.1.6	Indicates the manufacturer's catalogue number so that the medical device can be identified.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Temperature limit	5.3.7	Indicates the temperature limits to which the medical device can be safely exposed.
	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Consult instruction for use	5.4.3	Indicates the need for the user to consult the instructions for use.
IVD	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	*In vitro* diagnostic medical device	5.5.1	Indicates a medical device that is intended to be used as an *in vitro* diagnostic medical device.
Σ 96	ISO 15223-1: Medical Devices – Symbols to be used with medical device labels, labeling, and information to be supplied	Contains sufficient for 96 tests	5.5.5	Indicates the total number of IVD tests that can be performed with the IVD kit reagents.
Rx Only	Guidance for Industry and FDA on Alternative to Certain Prescription Device Labeling Requirements	Rx Only	N/A	Caution: Federal law prohibits dispensing without prescription.