510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number:

K091260

B. Purpose for Submission:

This is an application for an enzyme immunoassay for the qualitative detection of human IgG antibodies against EBV early antigen diffuse (EA-D) in human serum.

C. Measurand:

EBV early antigen diffuse (EA-D) IgG antibodies.

D. Type of Test:

ELISA assay designed to detect IgG reactivity to EBV EA-D using a 28 kd recombinant EBV early antigen.

E. Applicant:

Quest International, Inc.

F. Proprietary and Established Names:

SeraQuest EBV EA-D IgG Test

G. Regulatory Information:

1. Regulation section:

   21 CFR section 866.3235, Epstein-Barr virus serological reagents.

2. Classification:

   Class I

3. Product code:

   LSE

4. Panel:

   Microbiology (83)

H. Intended Use:

1. Intended use(s):

   The SeraQuest 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 other EBV-associated lymphomas.

2. Indication(s) for use:

   Same as intended use

3. Special conditions for use statement(s):

   For prescription use only

4. Special instrument requirements:

   Microwell reader capable of reading absorbance at 405 nm.

I. Device Description:

The SeraQuest EBV EA-D IgG test is an enzyme immunoassay intended for the qualitative detection of IgG antibodies against EBV EA-D. The test is an ELISA assay designed to detect IgG reactivity to EBV EA-D using a 28 kd *E. coli* expressed recombinant Epstein-Barr virus early antigen.

J. Substantial Equivalence Information:

1. Predicate device name(s):

   Trinity Biotech Captia EBV EA-D IgG

2. Predicate K number(s):

   K981120

3. <u>Comparison with predicate:</u>

Characteristic	SeraQuest EBV EA-D IgG (Investigational Device)	Trinity Biotech Captia EBV EA-D IgG (Predicate Device K981120)
Intended Use	Qualitative detection of IgG class antibodies to EBV EA-D in human serum. Intended to be used as an aid in the diagnosis of EBV-associated infectious mononucleosis.	Qualitative detection of IgG class antibodies to EBV EA-D in human serum. Intended to be used as an aid in the diagnosis of EBV-associated infectious mononucleosis.
Assay	ELISA	ELISA
Solid Phase	Plastic Microwell	Plastic Microwell
Antigen Used	Recombinant EA-D 28kd	Recombinant EA-D
Specimen Tested	Human serum	Human serum
Controls	1 human EBV EA-D IgG positive serum control 1 human EBV EA-D IgG negative serum control	1 human EBV EA-D IgG high positive serum or defibrinated plasma control 1 human EBV EA-D IgG low positive serum or defibrinated plasma control 1 human EBV EA-D IgG negative serum or defibrinated plasma control
Calibration	Human EBV EA-D IgG positive serum calibrator in every assay run to account for fluctuations in temperature and other testing conditions	Human EBV EA-D IgG positive serum or defibrinated plasma calibrator in every assay run to account for fluctuations in temperature and other testing conditions
Analyte Measured	IgG class antibodies to EBV EA-D	IgG class antibodies to EBV EA-D
# of Incubation Periods	Three	Four
Sample Dilution	1:51 in Diluent	1:21 in Diluent
Sample Incubation	30 +/- 5 minutes at RT	20 +/- 2 minutes at RT
Conjugate	Goat anti-human IgG	Goat anti-human IgG
Conjugate Label	Alkaline phosphatase	Horseradish Peroxidase
Conjugate Incubation	30 +/- 5 minutes at RT	20 +/- 2 minutes at RT
Substrate	p-Nitrophenyl phosphate	Tetramethylbenzidine (TMB)
Substrate Volume	100 μl	100 μl
Substrate Incubation	30 +/- 5 minutes at RT	20 +/- 2 minutes at RT
Stop Reagent	0.5 M Trisodium phosphate	1M H_2SO_4, 0.7M HCL
Stop Reagent Volume	100 μl	50 μl
Reading	Spectrophotometer at 405 nm	Spectrophotometer at 450 nm

K. Standard/Guidance Document Referenced (if applicable):

Not applicable.

L. Test Principle:

Diluted samples are incubated in wells coated with EBV Early Antigen D. Antibodies directed against the 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 goat antibodies to human IgG) is added and incubated. If IgG antibodies to EBV Early Antigen D are present, the conjugate will be immobilized on the wells. Residual conjugate is eliminated by washing and draining, and the enzyme substrate is added and incubated. In the presence of the enzyme, the substrate is converted to a yellow end-product which is read photometrically at 405 nm.

M. Performance Characteristics (if/when applicable):

1. <u>Analytical performance:</u>

   a. *Precision/Reproducibility:*

   A reproducibility panel of six (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 six 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 three (3) different clinical sites. In addition, one (1) SeraQuest human Anti-EA-D IgG positive serum control and one (1) SeraQuest human Anti-EA-D IgG negative serum control were also tested. Each of the six (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 three (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 calculated. Results can be found in Table 1.

Table 1: 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

b. *Linearity/assay reportable range:* Not applicable

c. *Traceability, Stability, Expected values (controls, calibrators, or methods):*

The calibrator index is traced to the in-house secondary standard for the SeraQuest EBV EA-D IgG test. The SeraQuest EBV EA-D IgG Secondary Standard was originally established in assays using the Trinity Biotech EBV EA-D IgG Calibrator with the SeraQuest EBV EA-D IgG Test reagents. A positive serum sample was adjusted by dilution to give an index value of 1.0 relative to the Trinity Calibrator. This became the first SeraQuest EBV EA-D IgG Calibrator, and aliquots were set aside as the in-house secondary standard.

d. *Detection limit:* Not applicable

e. *Analytical specificity:*
The cross-reactivity studies were 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 2.

Table 2: 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. 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.

Based on the Certificate of Analysis and the Antibody Dose Response Curves provided by the vendor of the *E. coli* expressed EBV EA-D recombinant antigen, Ross Southern Laboratories, two samples (designated as NC 1 and NC 2) that are both strongly positive for antibodies to *E. coli* tested negative in the antibody dose response experiment.

f. *Assay cut-off:*

The SeraQuest EBV EA-D IgG Secondary Standard / Calibrator was originally established in assays using the Trinity Biotech EBV EA-D IgG

Calibrator, with the SeraQuest EBV EA-D reagents. A positive serum sample was adjusted by dilution to give an index value of 1.0 relative to the Trinity Calibrator. This became the first SeraQuest EBV EA-D IgG Calibrator, and aliquots were set aside as the in-house secondary standard. The Calibrator / Secondary standard was then challenged in comparisons using the SeraQuest test and the Trinity test. The equivocal zone ($\geq$0.9 to < 1.1) was set 10% above, and 10% below, the cut-off value of 1.0.

The test cut-off was confirmed using a negative population for EBV EA-D IgG. The SeraQuest EBV EA-D IgG Test results were determined for this negative population, and the cut-off was confirmed at approximately the mean of the negative results plus two times the standard deviation (SD).

g. *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 3.

Table 3: 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

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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 interferents. However, the possibilities of interferences by the substances tested above can not be definitively ruled out. They have been addressed in the product package insert as limitations and warnings.

h. *Reaction Stability:*

A full SeraQuest ELISA plate containing ninety-one (91) samples was read immediately after the final reaction was stopped, and again after being allowed to stand at room temperature overnight, for 19 hours. No qualitative differences in the results were observed between the two readings. This data supports the statement in the "test procedure" section of the product package insert that states "Readings should be made within 2 hours after the reactions have been stopped at room temperature."

2. Comparison studies:

a. *Method comparison with predicate device:*

The SeraQuest EBV EA-D IgG Test was compared to the Trinity Biotech Captia EBV EA-D IgG Test in clinical trials at three (3) US testing sites.

b. *Matrix comparison:* Not applicable

3. Clinical studies:

a. b. *Clinical Sensitivity and specificity*

See Performance Characteristics below

c. *Other clinical supportive data (when a. and b. are not applicable)*:

Not applicable.

Performance Characteristics

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 4 below.

Table 4: EBV serological state characterization

EBV serological state	Specimen Group		EBV VCA IgG	EBV VCA IgM	EBV EBNA-1 IgG
	Prospectively Collected and Prospectively Tested	Prospectively Collected but Retrospectively Tested	EBV VCA IgG	EBV VCA IgM	EBV EBNA-1 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 4 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 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 50 sample results from Site A (Quest International) 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	0	0	0	0	0	0	0	0	2	2
EBV	2	0	0	0	0	0	0	2	4	8
Past Infection	11	2	0	0	2	0	1	2	14	32
Indeterminate	0	1	0	0	0	0	1	1	5	8
Overall	13	3	0	0	2	0	2	5	25	50

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	0/0	0%	-	2/2	100.0%	15.8 – 100
EBV Seronegative	2/2	100.0%	15.8 – 100	4/6	66.7%	22.3 – 95.7
Past infection	11/13	84.6%	54.5 – 98.1	14/17	82.4%	56.6 – 96.2
Indeterminate	0/1	0%	-	5/7	71.4%	29.0 – 96.3
Overall	13/16	81.2%	54.4 – 96.0	25/32	78.1%	60.0 – 90.7

Prospectively collected and prospectively tested 150 sample results from Site B (Mayo Clinic) are summarized in Tables 7-8.

Table 7: 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	2	0	0	2	0	0	1	0	4	9
EBV	1	0	0	0	0	1	2	1	21	26
Past Infection	8	0	1	6	0	2	8	5	53	83
Indeterminate	7	0	1	0	1	0	4	1	18	32
Overall	18	0	2	8	1	3	15	7	96	150

Table 8: 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	2/2	100.0%	15.8 – 100	4/7	57.1%	18.4-90.1
EBV Seronegative	1/2	50.0%	1.3-98.7	21/24	87.5%	67.6-97.3
Past infection	8/11	72.7%	39.0-94.0	53/72	73.6%	61.9-83.3
Indeterminate	7/8	87.5%	47.3-99.7	18/23	78.3%	56.3-92.5
Overall	18/23	78.3%	56.3-92.5	96/126	76.2%	68.8-83.6

Prospectively collected and prospectively tested 124 sample results from Site C (Doctor's Laboratory) are summarized in Tables 9-10.

Table 9: 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	1	0	0	0	0	0	2	1	2	6
EBV	1	0	1	0	0	1	0	0	8	11
Past Infection	12	5	8	0	2	5	2	3	48	85
Indeterminate	5	2	2	0	1	3	1	0	8	22
Overall	19	7	11	0	3	9	5	4	66	124

Table 10: 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	1/1	100.0%	-	2/5	40.0%	5.3-85.3
EBV Seronegative	1/3	33.3%	0.1-90.6	8/8	100.0%	63.1-100
Past infection	12/30	40.0%	22.7-59.4	48/53	90.6%	79.3-96.9
Indeterminate	5/12	41.7%	15.2-72.3	8/9	88.9%	51.8-99.7
Overall	19/46	41.3%	27.0-56.8	66/75	88.0%	78.4-94.4

Prospectively collected and prospectively tested 153 sample results from Site C (Doctor's Laboratory) are summarized in Tables 11-12.

Table 11: 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	9	0	1	0	0	1	0	0	3	14
EBV	0	2	0	0	1	3	0	1	8	15
Past Infection	34	6	2	1	4	5	6	4	49	111
Indeterminate	4	0	2	0	1	1	0	0	5	13
Overall	47	7	6	1	6	10	6	2	68	153

Table 12: 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	9/11	81.8%	48.2-97.7	3/3	100.0%	29.2-100
EBV Seronegative	0/5	0%	-	8/9	88.9%	51.8-99.7
Past infection	34/47	72.3%	57.4-84.4	49/60	81.7%	69.6-90.5
Indeterminate	4/7	57.1%	18.4-90.1	5/5	100.0%	47.8-100
Overall	47/70	57.1%	44.7-68.9	68/77	88.3%	79.0-94.5

Prospectively collected and prospectively tested 477 sample results from sites combined are summarized in Tables 13-14.

Table 13: 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	12	0	1	2	0	1	3	1	11	31
EBV	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 14: 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 (Quest International) are summarized in Tables 15-16.

Table 15: 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-			SeraQuest EBV EA-			SeraQuest EBV EA-			
	Pos	Equ	Neg	Pos	Equ	Neg	Pos	Equ	Neg	
	N	N	N	N	N	N	N	N	N	N
Acute	32	0	0	1	1	1	2	3	10	50
EBV	0	0	3	0	0	1	1	1	9	15
Overall	32	0	3	1	1	2	3	4	19	65

Table 16: 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 (the Trinity Captia EBV EA-D IgG Test) 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 17-18.

Table 17: 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 18: 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

4. Clinical cut-off**:**

See assay cut-off previously described in this document

5. Expected values/Reference range:

The clinical study included a total of 477 prospectively collected and prospectively tested specimens. The specimens were tested at three sites: Quest International, Inc. (Laboratory A) and two other clinical laboratories; one located in Midwestern U.S. (Laboratory B) and one located in the Southeastern region (Laboratory C). Of the 477 prospectively collected and prospectively tested specimens, patient demographic information were available for 153 samples that were 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 19 for serum samples from this particular patient cohort (N=153).

Table 19: 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%

N. Proposed Labeling:

Labeling is sufficient and it satisfies the requirements of 21 CFR section 809.10.

O. Conclusion:

The submitted information in this premarket notification is complete and supports a substantial equivalence decision.