510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY

A. 510(k) Number: K152353

B. Purpose for Submission: Clearance of New Device

C. Measurand: Herpes Simplex Virus-2 (HSV-2) type specific IgG antibodies to glycoprotein G (gG) 2 antigen.

D. Type of Test: Enzyme linked immunosorbent assay (ELISA)

E. Applicant: Quest International, Inc.

F. Proprietary and Established Names: SeraQuest HSV Type 2 Specific IgG

G. Regulatory Information:

1. Regulation section: 21 CFR§866.3305. Herpes simplex virus serological assays

2. Classification: Class II

3. Product code: MYF (Enzyme Linked Immunosorbent Assay, Herpes Simplex Virus, HSV-2)

4. Panel: Microbiology (83)

H. Intended Use:

The SeraQuest HSV Type 2 Specific IgG assay is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative detection of human IgG antibodies to type 2 herpes simplex virus (HSV) in human serum. The test is indicated for sexually active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-2 infection. The predictive value of a positive or negative result depends on the prevalence of HSV-2 infection in the population and the pre-test likelihood of HSV-2 infection.

The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for immunocompromised patients, pediatric patients or matrices other than human serum.

I. Device Description:

The SeraQuest HSV Type 2 Specific IgG test is a solid-phase enzyme-linked immunoassay (ELISA), which is performed in microwells, at room temperature, and in three thirty minute incubations. The test detects IgG antibodies which are directed against HSV-2 antigen,in human serum. The calibrators in the SeraQuest HSV Type 2 Specific IgG test set have been assigned index values based on an in-house standard. Test results are reported as index values. The following table shows the interpretation of results:

Index	Result	Interpretation
$\leq$ 0.9	Negative	No HSV-2 IgG antibodies detected. Patient is presumed not to have had a previous HSV-2 infection.
0.9 < X < 1.0	Equivocal	Obtain an additional sample for re-testing
$\geq$ 1.0	Positive	IgG antibody to HSV-2 detected.

Notes:

1. A single positive result only indicates previous immunologic exposure; the level of antibody response may not be used to determine active infection or disease stage.

2. When equivocal results are obtained, another specimen should be obtained ten to fourteen days later, and tested in parallel with the initial specimen. If the second specimen is also equivocal, the patient is negative for primary or recent infection, and equivocal for antibody status. If the second sample is positive, the patient can be considered to have previous experience with HSV-2 infection.

3. Values obtained with different manufacturer's assay methods may not be used interchangeably. The magnitude of the reported IgG index value cannot be correlated to an endpoint titer. The magnitude of results above the cut-off is not an indicator of total antibody present.

J. Substantial Equivalence Information:

1. Predicate device name(s): Focus HerpeSelect® 1 and 2 Immunoblot IgG

2. Predicate 510(k) number(s): K000238

3. Comparison with predicate:

Similarities		
Item	**SeraQuest HSV 2 IgG**	**Focus HerpeSelect 2 and 2 Immunoblot IgG (Predicate)**
Intended Use	The SeraQuest HSV Type 2 Specific IgG assay is an enzyme-linked immunosorbent assay (ELISA) intended for the qualitative detection of human IgG antibodies to type 2 herpes simplex virus (HSV) in human serum. The test is indicated for sexually	Focus Diagnostics' HerpeSelect 1 and 2 Immunoblot IgG test is intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-1 and HSV-2 in human sera. The test is indicated for testing sexually active adults or expectant mothers for aiding

Similarities		
Item	**SeraQuest HSV 2 IgG**	**Focus HerpeSelect 2 and 2 Immunoblot IgG (Predicate)**
	active individuals and expectant mothers as an aid in the presumptive diagnosis of HSV-2 infection. The predictive value of a positive or negative result depends on the prevalence of HSV-2 infection in the population and the pre-test likelihood of HSV-2 infection. The test is not FDA cleared for screening blood or plasma donors. The performance of this assay has not been established for immunocompromised patients, pediatric patients or matrices other than human serum.	in the presumptive diagnosis of HSV-1 and HSV-2 infection. The predictive value of a positive or negative result depends on the population's prevalence and the pretest likelihood of HSV-1 and HSV-2 infection. The performance of this assay has not been established for use in a pediatric population, for neonatal screening, for testing of immunocompromised patients, for use by a point of care facility or for use with automated equipment.
Sample Matrix	Human Serum	Human Serum
Incubation Periods	Three	Three
Incubation Temperature	Room Temperature	Room Temperature
Enzyme-Labeled Conjugate	Alkaline phosphatase conjugated goat anti-human IgG	Alkaline phosphatase conjugated goat anti-human IgG
Controls	Positive and negative controls included with kit	Positive and negative controls included with kit

Differences		
Item	**SeraQuest HSV 2 IgG**	**Focus HerpeSelect 2 and 2 Immunoblot IgG (Predicate)**
Type of Assay	Enzyme linked immunoassay (ELISA)	Nitrocellulose immunoblot
Solid Phase	Polystyrene Microwells	Nitrocellulose membrane
Calibrator	Included with reagent kit	N/A
Sample Dilution	1:51	1:101
Antigen	Recombinant HSV gG 2	Recombinant HSV gG1 and HSV gG2 (separate bands)

Differences		
Item	**SeraQuest HSV 2 IgG**	**Focus HerpeSelect 2 and 2 Immunoblot IgG (Predicate)**
Sample Volume	100 uL	2000 uL
Sample incubation duration	30 minutes	60 minutes
Washing Steps	Two	Four
Cycles per wash step	Four	Three
Conjugate Volume	100 uL	2000 uL
Enzyme substrate	p-nitrophenyl phosphate	Bromo-chloro-indolyl phosphate and nitro blue tetrazolium
Substrate Volume	100 uL	2000 uL
Substrate Incubation	30 minutes	5 to 30 minutes
Stop Reagent	0.5 M Trisodium Phosphate	DI Water
Stop Reagent Volume	100 uL	5x 2000 uL
Drying Step	None	Air dry, 10 minutes
Readout	Spectrophotometric	Visual
Unit of Measure	Index value	Positive or negative

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

Guidance for Industry and FDA Staff - Class II Special Controls Guidance Document: Herpes Simplex Virus Types 1 and 2 Serological Assays (issued August 9, 2011).

L. Test Principle:

Diluted patient serum samples are incubated in antigen-coated wells. HSV IgG Type 2 antibodies, if present in the patient sample, are immobilized in the wells by binding to the antigen. Residual sample is eliminated by washing and draining, and conjugate is added and incubated. If IgG antibodies to HSV Type 2 are present in the sample, the conjugate will be immobilized in the wells. Residual conjugate is eliminated by washing and draining, and the substrate is added and incubated. In the presence of the conjugate, the substrate is converted to a yellow product which is read photometrically. Test results are obtained after one and one half hours incubation time.

M. Performance Characteristics (if/when applicable):

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

   a. Precision/Reproducibility:

   Six serum specimens (2 negative, 1 equivocal, and 3 positive) and the SeraQuest HSV Type 2 Specific IgG Positive and Negative Controls were assayed in triplicate at one internal and two external laboratories. The results are summarized in the following table:

Panel Member	Sample N	Mean Index	Intra-assay		Inter-assay		Inter-laboratory		Total	
			SD	CV%	SD	CV%	SD	CV %	SD	CV %
Positive Control	27	2.0	0.20	9.9	0.27	13.7	0.31	15.4	0.26	13.0
Negative Control	27	0.3	0.04	13.5	0.08	21.2	0.15	48.2	0.09	27.6
Negative Sample #1	27	0.3	0.08	26.7	0.10	31.2	0.11	35.9	0.10	31.3
Negative Sample #2	27	0.4	0.04	9.6	0.06	14.4	0.09	21.6	0.06	15.2
Positive Sample #1	27	1.8	0.15	8.3	0.19	10.7	0.23	12.4	0.19	10.5
Positive Sample #2	27	2.1	0.12	5.7	0.18	8.8	0.21	10.0	0.17	8.1
Positive Sample #3	27	2.8	0.16	5.6	0.25	9.0	0.32	11.4	0.24	8.7
Positive Sample #4	27	3.5	0.27	7.8	0.38	10.7	0.42	11.9	0.36	10.1

b. Linearity/assay reportable range:
Not Applicable.

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

Controls:

Positive Control	Composed of human serum reactive for HCV IgG Type 2 antibodies. The Positive Control is assigned an Index Value between 1.1 and 3.3.
Negative Control	Composed of human serum non-reactive for HCV IgG Type 2 antibodies.

Calibrators:
The Calibrator is composed of human serum strongly reactive for HCV IgG Type 2 antibodies. The Calibrator is assigned an Index Value between 1.1 and 2.1.

Analyte Stability
If serum specimens are not tested within 8 hours, they should be stored at 2 to 8^{o}C for up to 48 hours. Beyond 48 hours, specimens should be stored at -20^{o}C or below. Serum specimens stored for up to seventeen months at -20^{o}C showed no significant changes in index values upon retest. Samples may be frozen and thawed once. Samples that are hemolyzed, icteric, lipemic, grossly contaminated or contain visible particulate matter should not be used. Samples should not be heat-inactivated before testing.

d. Detection limit:
Not Applicable.

e. Analytical specificity:

Endogenous Interfering Substances
The effects of elevated levels of the following substances on the SeraQuest HSV Type 2 Specific IgG test were examined: hemoglobin 18 g/dL, glucose 800 mg dL,

cholesterol 2,720 mg/dL, globulin 28 g/dL, unconjugated bilirubin 20 mg/dL, conjugated bilirubin 20 mg/dL, human albumin 12 g/dL and ascorbic acid 3mg/dL.. Samples that were negative, weakly positive, and moderately positive for antibodies to Type 2 HSV IgG were tested with and without the addition of elevated levels of interfering substances well above physiological concentrations. No interference was observed.

Cross-reactivity

A cross-reactivity study was performed to determine if samples from various disease states and other potentially cross-reacting agents interfere with the test results. The samples were determined to be positive for IgG antibodies directed against taxonomically related viruses and other related pathogens by other legally marketed devices. Human Papilloma Virus, *Chlamydia trachomitis*, and *Neisseria gonorrhea* samples were from individual patients with confirmed sexually transmitted infections. The samples were tested for Type 2 HSV antibody by another legally marketed device. Only those samples that tested negative for Type 2 HSV antibody by the legally marketed device were included in the study. The following table summarizes the results:

Samples	Number of Samples	Number of Samples Testing Positive in the SeraQuest HSV Type 2 Specific IgG Test
HSV 1 IgG	9	0/9
CMV IgG	11	0/11
EBV EBNA IgG	14	0/14
EBV VCA IgG	17	0/17
VZV IgG	21	0/21
Measles IgG	19	0/19
Rubella IgG	18	0/18
Toxoplasma IgG	6	0/6
Syphilis IgG	4	0/4
Human Papilloma Virus	7	0/7
Chlamydia trachomitis	8	1/8
Neisseria gonorrhea	7	0/7

f. Assay cut-off:

The SeraQuest HSV Type 2 Specific IgG test cut-off value was initially obtained when 138 serum specimens shown to be negative by another commercial test were assayed by the SeraQuest HSV Type 2 Specific IgG test. The cut-off value was evaluated using Receiver Operator Characteristic (ROC) Curve analysis using four different hypothetical positive cut-off Index Values (0.9, 1.0, 1.1, 1.2). The results of the ROC curve are supportive of 1.0 as a cut-off value for separating reactive and equivocal test results. The cut-off value was subsequently challenged using a panel of 100 masked and coded serum specimens, which had been well characterized by EIA test and western blot.

2. Comparison studies:
   a. *Method comparison with predicate device:*

Sexually Active Adults

One hundred and sixty four serum samples (164), from sexually active adults that were submitted for HSV serology to a clinical laboratory in the Southeastern United States, were prospectively collected, masked, archived, and tested using the SeraQuest HSV Type 2 Specific IgG test and an FDA cleared HSV2 immunoblot test. The results are shown in the following table:

SeraQuest HSV Type 2 Specific IgG Result						
	Positive	Equivocal	Negative		% Agreement	95% C.I.
HSV 2 Immunoblot Result						
Positive	56	0	5	Sensitivity	91.8	82.2to 96.5
Negative	6	0	97	Specificity	94.2	87.9 to 97.3

Expectant Mothers

Two hundred and forty-two (242) serum samples, from expectant mothers, that were submitted for HSV serology to clinical laboratories in the Northeastern and Southeastern United States, were prospectively collected, masked, archived, and tested at Quest International, Inc. using the SeraQuest HSV Type 2 Specific IgG test and a legally marketed, HSV 2 Immunoblot test. One hundred and ninety-eight (82%) of the specimens were obtained during the first trimester, nineteen (8%) during the second trimester and twenty-five (10%) during the third trimester of pregnancy. The results of this comparative test are shown in the following table:

Seraquest HSV Type 2 Specific IgG Result						
	Positive	Equivocal	Negative		% Agreement	95% C.I.
HSV 2 Immunoblot Result						
Positive	87	0	1	Sensitivity	98.9	93.8 to 99.8
Negative	0	1	153	Specificity	99.4	96.4 to 99.9

   b. *Matrix comparison:*
   Not Applicable.

3. Clinical studies:

   a. *Clinical Sensitivity:*
   Not Applicable.

b. Clinical specificity:
Not Applicable.

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

Agreement with CDC Panel
The following information was obtained with the Centers for Disease Control and Prevention (CDC) serum panel for HSV serology assays, which was tested in-house by the SeraQuest HSV Type 2 Specific IgG test. The results are presented as a means to convey further information on the performance of this assay with a masked, well characterized serum panel. This does not imply an endorsement by the CDC.

The panel consists of 30 HSV-2 IgG positive and 70 HSV-2 IgG negative samples. The SeraQuest HSV Type 2 Specific IgG test demonstrated 100% total agreement with the CDC consensus results. The results of this study are shown in the following table:

		SeraQuest HSV Type 2 Specific IgG			
		Positive	Equivocal	Negative	Total
CDC HSV 2 Result	Positive	30	0	0	30
	Negative	0	0	70	70
	Total	30	0	70	100

4. Clinical cut-off**:**
Not Applicable.

5. Expected values/Reference range:
The performance of the SeraQuest HSV Type 2 Specific IgG test was assessed with masked, archived, unselected sera from 164 sexually active adults and from 242 expectant mothers. The reference method was a commercial Immunoblot test. The observed prevalence and the hypothetical predictive values for the two populations are shown in the tables below. The positive predictive value will decrease proportionally with the prevalence of HSV infection as shown below. The calculations were based on the SeraQuest HSV Type 2 Specific IgG test having a sensitivity of 91.8% and a specificity of 94.2% for the sexually active adults; and a sensitivity of 98.9% and a specificity of 99.4% for the expectant mothers.

The following table shows the observed prevalence of HSV 2 IgG Antibodies in Sexually Active Adults and Expectant Mothers:

Population	Sero-Status	Observed Prevalence
		SeraQuest HSV Type 2 Specific IgG
Sexually Active Adults	Negative	62.2% (102/164)
	Positive	37.8% (62/164)
Expectant Mothers	Negative	63.2% (153/242)
	Positive	35.8% (87/242)

The following table shows the observed prevalence of HSV 2 IgG antibodies in sexually active adults (N=164) stratified by age:

Age in Years	Gender	Positive		Equivocal		Negative		Total
		N	%	N	%	N	%	
18-20	F	14	78	0	0	4	22	18
	M	4	80	0	0	1	20	5
21-30	F	28	67	0	0	14	33	42
	M	8	80	0	0	2	20	10
31-40	F	14	70	0	0	6	30	20
	M	6	60	0	0	4	40	10
41-50	F	11	50	1	5	10	45	22
	M	8	62	0	0	5	38	13
51-60	F	4	36	1	9	6	55	11
	M	1	17	0	0	5	83	6
61-70	F	2	50	0	0	2	50	4
	M	1	33	1	33	1	33	3
71-80	F	1	100	0	0	0	0	1
	M	0	0	0	0	0	0	0
81-89	F	0	0	0	0	0	0	0
	M	0	0	0	0	0	0	0
Total		102	62	3	2	59	36	164

The following table shows the observed prevalence of HSV 2 IgG antibodies in expectant mothers (N=242) stratified by age:

Age in Years	Positive		Equivocal		Negative		Total
	N	%	N	%	N	%	
18-20	16	72	1	5	5	23	22
21-30	95	64	2	1	51	35	148
31-40	38	58	0	0	28	42	66
41-50	4	67	0	0	2	33	6
Total	153	63	3	1	86	36	242

The following table shows the prevalence versus the hypothetical predictive values:

Prevalence	Sexually Active Adults		Expectant Mothers	
	PPV	NPV	PPV	NPV
50%	94.0%	91.9%	99.3%	98.9%
40%	91.3%	94.5%	99.0%	99.2%
30%	87.2%	96.4%	98.6%	99.5%
25%	84.0%	97.1%	98.2%	99.6%
20%	79.8%	97.8%	97.6%	99.7%
15%	73.6%	98.4%	96.6%	99.8%
10%	63.7%	99.0%	94.8%	99.8%
5%	45.4%	99.5%	89.8%	99.9%

N. Proposed Labeling:

The labeling is sufficient and it satisfies the requirements of 21 CFR Part 809.10.

O. Conclusion:

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