

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION
DECISION SUMMARY
DEVICE ONLY TEMPLATE**

A. 510(k) Number: K033106

B. Purpose for Submission: This premarket notification is for new device clearance.

C. Analyte: Captia™ HSV 2 Type Specific IgG

D. Type of Test: Enzyme-linked Immunosorbent Assay (ELISA)

E. Applicant: Trinity Biotech USA

F. Proprietary and Established Names: Trinity Biotech Captia™ Herpes Simplex Virus (HSV) 2 Type Specific IgG

G. Regulatory Information:

1. Regulation section: 21 CFR 866.3305
2. Classification: III
3. Product Code: MYF
4. Panel: 83

H. Intended Use:

1. Intended use and indications for use: The Trinity Biotech Captia™ Herpes Simplex Virus (HSV) 2 Type Specific IgG kit is an Enzyme-linked Immunosorbent Assay (ELISA) intended for qualitatively detecting the presence or absence of human IgG class antibodies to HSV-2 in human sera. In conjunction with the Trinity Biotech Captia™ Herpes Simplex Virus (HSV) 1 Type Specific IgG kit, the test is indicated for testing sexually active adults or expectant mothers for aiding in the presumptive diagnosis of HSV infection.
2. Special condition for use statement(s): The performance of this assay has not been established for use in a pediatric population, for neonatal screening, for testing of immunocompromised patients, or for use with automated equipment. This device is for prescription use only.
3. Special instrument requirements: N/A

K. Device Description: Enzyme-Linked Immunosorbent Assays (ELISA) rely on the ability of biological materials (i.e., antigens) to adsorb to plastic surfaces such as polystyrene (solid phase). When antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG

conjugated with horseradish peroxidase, which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of chromogen/substrate, Tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.

I. Substantial Equivalence Information:

1. Predicate device name(s): Focus Technologies HerpeSelect® 2 ELISA IgG
2. Predicate K number(s): K0214856
3. Comparison with predicate:

Similarities		
Item	Device	Predicate
<ol style="list-style-type: none"> 1. Both utilize the type-specific immunoglobulin g (HSV gG2 IgG). 2. Both use recombinant antigens. 3. Both did cross-reactivity testing on <u>only</u> taxonomically related viruses 4. Both were adequately tested with sexually active adults and expectant mothers 5. Both are intended for presumptive diagnosis. 6. Both are limited with testing on pediatric, neonatal or immunocompromised populations 7. Both are compared to Western blot. 8. Both incubate serum for 30 minutes 9. The substrate is TMB and the stop is H₂SO₄ for both. 10. Both use a high and low positive and negative control along with a cutoff calibrator. 	Captia™ HSV 2 Type-Specific IgG	Focus Technologies HerpeSelect® 2 ELISA IgG

11. The index/ISR values for interpretation of results are the same for both (adjusted per lot number for each calibrator).		
Differences		
Item	Device	Predicate
<ol style="list-style-type: none"> 1. Trinity did not compare their device to automated methodology where Focus did. 2. Trinity incubates substrate for 15 minutes and Focus for 10 minutes 3. Trinity recommends washing 3X and Focus 5X. 4. Trinity uses air as a blank and Focus uses the sample diluent as such. 5. Both assays are to be read at 450 nm but Trinity also incorporates a dual wavelength of 650 nm. 	Captia™ HSV 2 Type-Specific IgG	Focus Technologies HerpeSelect® 2 ELISA IgG

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

L. Test Principle: HSV 1 and HSV 2 have approximately 50% sequence homology and show considerable cross-reactivity. The Trinity Biotech HSV 2 Type Specific IgG ELISA uses a recombinant glycoprotein g, which is type specific for HSV 2. This allows for a rapid sero-diagnosis of HSV 2 infection than virus isolation techniques.

The Trinity Biotech HSV 12Type Specific IgG kit utilizes the ELISA technology where a purified recombinant HSV 1 antigen is bound to the wells of a microplate. A peroxidase coupled anti-human IgG conjugate is used as the detection system. When rHSV1 antigens bound to the solid phase are brought into contact with a patient's serum, antigen specific antibody, if present, will bind to the antigen on the solid phase forming antigen-antibody complexes. Excess antibody is removed by washing. This is followed by the addition of goat anti-human IgG conjugated with horseradish peroxidase, which then binds to the antibody-antigen complexes. The excess conjugate is removed by washing, followed by the addition of chromogen/substrate, Tetramethylbenzidine (TMB). If specific antibody to the antigen is present in the patient's serum, a blue color develops. When the enzymatic reaction is stopped with 1N H₂SO₄, the contents of the wells turn yellow. The color, which is indicative of the concentration of antibody in the serum, can be read on a suitable spectrophotometer or ELISA microwell plate reader.

M. Performance Characteristics (if/when applicable):1. Analytical performance:a. **Precision/Reproducibility:**

Table 1

HSV 2 Type Specific IgG Intra and Inter Assay Precision

Study Site 1

Serum ID	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%
1	2.95	0.071	2%	2.95	0.089	3%	3.06	0.084	3%	2.99	0.095	3%
2	1.78	0.022	1%	1.78	0.042	2%	1.90	0.030	2%	1.82	0.063	3%
3	2.12	0.031	1%	2.10	0.040	2%	2.27	0.089	4%	2.16	0.095	4%
4	2.49	0.050	2%	2.53	0.039	2%	2.61	0.075	3%	2.54	0.074	3%
5	0.17	0.003	2%	0.19	0.075	4%	0.20	0.006	3%	0.19	0.044	24%
6	0.30	0.013	4%	0.35	0.053	15%	0.54	0.054	10%	0.40	0.115	29%

Table 2

HSV 2 Type Specific IgG Intra and Inter Assay Precision

Study Site 2

Serum ID	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%
1	3.56	0.073	2%	3.43	0.138	4%	3.35	0.111	3%	3.45	0.107	3%
2	2.61	0.131	5%	2.31	0.188	8%	2.44	0.124	5%	2.46	0.148	6%
3	2.84	0.092	3%	2.86	0.169	6%	2.74	0.075	3%	2.81	0.112	4%
4	3.63	0.113	3%	3.12	0.246	8%	3.71	0.130	4%	3.49	0.163	5%
5	0.68	0.035	5%	0.39	0.087	22%	0.65	0.032	5%	0.57	0.051	11%
6	0.74	0.034	5%	0.77	0.114	15%	0.69	0.062	9%	0.73	0.070	9%

Table 3

HSV 2 Type Specific IgG Intra and Inter Assay Precision

Study Site 3

Serum ID	Assay 1 (n=10)			Assay 2 (n=10)			Assay 3 (n=10)			Inter Assay (n=30)		
	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%	\bar{X}	S.D.	CV%
1	3.03	0.062	2%	3.2	0.093	3%	2.98	0.048	2%	3.07	0.120	4%
2	1.84	0.047	2%	1.91	0.058	3%	1.79	0.037	2%	1.85	0.067	4%
3	2.17	0.050	2%	2.25	0.096	4%	2.18	0.054	2%	2.20	0.077	4%
4	2.46	0.070	3%	2.52	0.099	4%	2.48	0.054	2%	2.48	0.078	3%
5	0.29	0.013	4%	0.36	0.018	5%	0.38	0.091	23%	0.34	0.065	19%
6	0.52	0.015	3%	0.57	0.020	3%	0.56	0.021	4%	0.55	0.027	5%

Table 4
HSV 2 Type Specific IgG Inter Site Precision

Sample ID	Site 1 \bar{X}	Site 2 \bar{X}	Site 3 \bar{X}	\bar{X}	S.D.	C.V.
1	2.99	3.45	3.07	3.17	0.246	8%
2	1.82	2.46	1.85	2.04	0.361	18%
3	2.16	2.81	2.20	2.39	0.364	15%
4	2.54	3.49	2.48	2.84	0.567	20%
5	0.19	0.57	0.34	0.37	0.191	52%
6	0.40	0.73	0.55	0.56	0.165	30%

\bar{X} = Mean ISR Value
S.D. = Standard Deviation
C.V. = Coefficient of Variation

- b. **Linearity/assay reportable range:** N/A
- c. **Traceability, stability, expected values (controls, calibrators, or method):** There is no guidance document utilized but the sponsor has compared their device to the CDC serum panel of well characterized HSV results.

The panel consisted of 36.0% HSV 2 positive and 64.0% HSV 2 negative specimens. The Trinity Biotech HSV 2 Type Specific IgG ELISA demonstrated 92.0% total agreement with the CDC results. Of the results obtained by Trinity Biotech, there was 94.4% agreement with all HSV 2 positive specimens (This includes sera that are positive for HSV 2 only and sera that are positive for both HSV 1 and HSV 2) and 90.6% agreement with the HSV 2 negative specimens. There was 83.3% agreement with the specimens that were HSV 1 positive only and 100.0% agreement with the specimens that were HSV negative for both 1 and 2. (Please provide FDA with a copy of the the data sheet with results which you would have received from the CDC.)

- d. **Type specificity with HSV 1 Western blot positives**

An outside investigator at a Pacific Northwest University assessed the type specificity using HSV 1 Western Blot positive and HSV 2 Western Blot negative sera from the above described populations (n = 292): expectant mothers, sexually active adults, low prevalence persons, and HSV 1 culture positives. Of 292 HSV 1 Western Blot positive and HSV 2 Western Blot negative samples: ELISA was 265 negative, 23 positive and 4 equivocal.

Characteristic	% (EL/WB)	95% Confidence Interval (CI)
Type-specificity relative to WB	92.0% (265/288)	88.3-94.9%
Type cross-reactivity relative to WB	8.0% (23/288)	5.13-11.7%

- e. **Detection limit:** N/A
- f. **Analytical specificity:** Because there was no interference testing performed, the sponsor has placed the following in the Limitations section of their PI: Icteric, lipemic, hemolyzed or heat inactivated

sera may cause erroneous results and are not recommended to be used, since they could possibly affect test results.

- g. **Analytical cut-off:** Ninety-five HSV Type 2 negative sera were assayed by the HSV 2 IgG Type Specific ELISA test. The mean and standard deviation of the optical density readings for the sera was 0.147 and 0.093 respectively. The positive threshold for the assay was determined by adding the mean and 2.5 standard deviations $0.147 + 2.5 (0.093) = 0.380$. A positive serum was titrated to give a constant ratio of the threshold value to obtain a calibrator sera. On all subsequent assays, this sera was run and the assay calibrated by multiplying the O.D. value for the calibrator by the ratio to the cut off to obtain the cut off O.D. This value was then divided into the O.D. for the patient sera to obtain an index value. By definition, the cut off index is equal to 1.00. To account for inherent variation in the immunoassay, values of 0.91 – 1.09 were considered equivocal. Therefore values ≤ 0.90 are considered negative and the values ≥ 1.10 are considered positive. Further analytical validation was performed using levels 15% above and 30% below the cut off.

2. Comparison studies:

- a. **Method comparison with predicate device:** % Agreement Positive and % Agreement Negative with Alternate HSV 2 Type Specific IgG ELISA.

An outside investigator at a Pacific Northwest University assessed the % agreement positive and % agreement negative of the Trinity Biotech Captia™ HSV 2 Type Specific IgG kit and alternate HSV 2 type specific IgG ELISA test with 200 prospective, unselected, sequentially submitted specimens.

Prospectively Collected, Sequential Sera		Alternate HSV 2 Type Specific IgG		
		+	-	E
Trinity Biotech Captia™ HSV 2 Type Specific	+	68	10	2
	-	2	117	0
	E	0	1	0

Characteristic	% (TBU ELISA / Alt. ELISA)	95% Confidence Interval (CI)
Percent Positive Agreement	97.14 % (68 / 70)	90.1 – 99.7 %
Percent Negative Agreement	92.13 % (117 / 127)	86.0 – 96.2 %
Percent Agreement	92.50 % (185 / 200)	87.9 – 95.7 %

- b. **Matrix comparison:** N/A since this is intended for serum only.

3. Clinical studies:

a. **Clinical sensitivity:**

i) % Agreement Positive and % Agreement Negative with Expectant Mothers (n = 210) †

An outside investigator assessed the % agreement positive and % agreement negative with consented, coded, unselected, banked and masked sera from expectant mothers (n = 210). The reference method was an HSV 2 Western Blot (WB) from a Pacific Northwest University. Of 43 WB positives: Trinity ELISA was 43 positive. Of 165 WB negatives: Trinity ELISA was 151 negative, 13 positive and 1 equivocal.

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
% agreement positive to WB	100.00% (43/43)	91.8-100.0%
% agreement negative to WB	92.07% (151/164)	86.8-95.7%

+

* Excludes one ELISA equivocal, one atypical Western Blot and one sample that was both atypical Western Blot and ELISA equivocal.

† The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.

ii) % Agreement Positive and % Agreement Negative with Sexually Active Adults (n = 198)†

An outside investigator assessed the % agreement positive and % agreement negative with consented, unselected and masked sera from sexually active adults over the age of 14 (n = 198). The reference method was an HSV 2 Western Blot (WB) from a Pacific Northwest University. Of 61 WB positives: Trinity ELISA was 59 positive and 2 negative. Of 134 WB negatives: Trinity ELISA was 121 negative and 13 positive.

Characteristic	% (EL/WB)*	95% Confidence Interval (CI)
% agreement positive to WB	96.72% (59/61)	88.7-99.6%
% agreement negative to WB	90.30% (121/134)	84.0-94.7%

* Excludes one ELISA equivocal, one atypical Western Blot and one sample that was both atypical Western Blot and ELISA equivocal.

† The word "% agreement" refers to comparing this assay's results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay's accuracy in predicting disease.

iii) % Agreement Positive and % Agreement Negative with a Low Prevalence Population (n = 184)†

An outside investigator assessed the % agreement positive and % agreement negative with unselected, banked and masked sera from a low prevalence population (n = 184). The reference method was an HSV 2 Western Blot (WB) from a Pacific Northwest University. Of 179 WB negatives: Trinity ELISA was 163 negative, 14 positive and 2 equivocal. Of 4 WB positives: Trinity ELISA was 4 positive.

<i>Characteristic</i>	<i>% (EL/WB)*</i>	<i>95% Confidence Interval (CI)</i>
% agreement positive to WB	100.00% (4/4)	39.8-100.0%
% agreement negative to WB	92.09% (163/177)	87.1-95.6%

* Excludes two ELISA equivocal and one atypical Western Blot.

† The word “% agreement” refers to comparing this assay’s results with those of a similar assay. No attempt was made to correlate the assay results to disease presence or absence. No judgment can be made on the similar assay’s accuracy in predicting disease.

iv) % Agreement Positive with Culture Positives (n = 56)†

An outside investigator assessed the % agreement positive using unselected, retrospective and masked sera from patients that were at least six weeks but not more than one year post clinical presentation and culture HSV 2 positive (n = 56). Reference methods included culture (infection) and an HSV 2 Western Blot (WB) (antibody) from a Pacific Northwest University. Of 56 culture positives: 1) Trinity ELISA was 56 positive and, 2) WB was 55 positive and 1 negative.

<i>Characteristic</i>	<i>% (EL/WB or Culture)</i>	<i>95% Confidence Interval (CI)</i>
% agreement positive to culture	100.00% (56/56)	93.6-100.0%
% agreement positive to WB	98.2% (55/56)	90.4-100.0%

† The word “% agreement” refers to comparing this assay’s results with culture, considered the gold standard.

b. **Clinical specificity:** Cross-reactivity

Because minimal cross-reactivity performance testing was done, each laboratory should consider performing testing on taxonomically related viruses and viruses which could cause a syndrome similar to HSV such as HPV and gonorrhea. The levels tested should exceed ISR values of 3.10.

The above bolded and boxed warning is warranted in this section because of the limited cross-reactivity performed. The sponsor states that a study was performed to determine the cross-reactivity of the Trinity Biotech HSV 2 Type Specific IgG ELISA test with 44 sera containing IgG antibody to taxonomically similar viruses including Cytomegalovirus (CMV), Varicella-Zoster Virus (VZV), and Epstein-Barr Virus (EBV). Of the 44 sera, 12 tested positive for CMV IgG by ELISA, 37 tested positive for VZV IgG by ELISA, and 42 tested positive for EBV IgG by ELISA. All 44 sera were negative by the Trinity Biotech HSV 2 Type Specific IgG ELISA indicating that antibodies to these viruses do not cross-react with the Trinity Biotech HSV 2 Type Specific IgG ELISA.

A study was performed to determine the cross-reactivity of the Trinity Biotech HSV 2 Type Specific IgG ELISA test with 44 sera containing IgG antibody to related pathogens including Measles, Rubella and Syphilis. Of the 44 sera, the same 25 tested positive for Measles IgG and Rubella IgG by ELISA, and 19 tested positive for Syphilis IgG by ELISA. All 44 sera were negative by the Trinity Biotech HSV 2 Type Specific IgG ELISA indicating that antibodies to these related

pathogens do not cross-react with the Trinity Biotech HSV 2 Type Specific IgG ELISA.

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

1. Clinical cut-off: Only an analytical cutoff was performed.
2. Expected values/reference range: Expected values were established when clinical specimens were compared to culture and WB.

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