

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

A. 510(k) Number: K033105

B. Purpose for Submission: This submission follows a Pre-IDE protocol with a premarket notification submitted and given a final review on July 9, 2004 after response to a desk hold on June 23, 2004.

C. Analyte: Captia™ HSV 1 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) 1 Type Specific IgG

G. Regulatory Information:

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

H. Intended Use:

1. Intended use and Indications for Use: The Trinity Biotech Captia™ Herpes Simplex Virus (HSV) 1 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-1 in human sera. In conjunction with the Trinity Biotech Captia™ Herpes Simplex Virus (HSV) 2 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.
3. Special instrument Requirements: N/A

I. 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.

J. Substantial Equivalence Information:

1. Predicate device name(s): Focus Technologies HerpeSelect® 1 ELISA IgG
2. Predicate K number(s): K021429
3. Comparison with predicate:

| Similarities | | |
|---|---------------------------------|---|
| Item | Device | Predicate |
| 1. Both utilize the type-specific immunoglobulin g (HSV gG1 IgG). | Captia™ HSV 1 Type-Specific IgG | Focus Technologies HerpeSelect® 1 ELISA 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 H ₂ SO ₄ for both. | | |
| 10. Both use a high and low positive and negative control along with a cutoff calibrator. | | |

| <p>11. The index/ISR values for interpretation of results are the same for both (adjusted per lot number for each calibrator).</p> | | |
|---|--|--|
| Differences | | |
| Item | Device | Predicate |
| <p>1. Trinity did not compare their device to automated methodology where Focus did.</p> <p>2. Trinity incubates substrate for 15 minutes and Focus for 10 minutes</p> <p>3. Trinity recommends washing 3X and Focus 5X.</p> <p>4. Trinity uses air as a blank and Focus uses the sample diluent as such.</p> <p>5. Both assays are to be read at 450 nm but Trinity also incorporates a dual wavelength of 650 nm.</p> | <p>Captia™ HSV 1 Type-Specific IgG</p> | <p>Focus Technologies HerpeSelect® 1 ELISA IgG</p> |

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

K. **Test Principle:** HSV 1 and HSV 2 have approximately 50% sequence homology and show considerable cross-reactivity. The Trinity Biotech HSV 1 Type Specific IgG ELISA uses a recombinant glycoprotein g, which is type specific for HSV 1. This allows for a rapid and less expensive sero-diagnosis of HSV 1 infection than virus isolation techniques (9, 10, 11, 12).

The Trinity Biotech HSV 1 Type 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 1 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 | 2.84 | 0.109 | 4% | 2.71 | 0.239 | 9% | 2.81 | 0.152 | 5% | 2.79 | 0.179 | 6% |
| 2 | 5.28 | 0.113 | 2% | 5.08 | 0.280 | 6% | 5.36 | 0.146 | 3% | 5.24 | 0.222 | 4% |
| 3 | 3.54 | 0.077 | 2% | 3.40 | 0.073 | 2% | 3.62 | 0.096 | 3% | 3.52 | 0.122 | 3% |
| 4 | 2.27 | 0.117 | 5% | 2.11 | 0.107 | 5% | 2.23 | 0.067 | 3% | 2.20 | 0.118 | 5% |
| 5 | 0.15 | 0.039 | 26% | 0.12 | 0.027 | 23% | 0.12 | 0.013 | 11% | 0.13 | 0.032 | 24% |
| 6 | 0.09 | 0.036 | 42% | 0.05 | 0.015 | 32% | 0.03 | 0.007 | 21% | 0.06 | 0.031 | 55% |

Table 2
HSV 1 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 | 3.40 | 0.109 | 3% | 3.32 | 0.196 | 6% | 2.75 | 0.118 | 4% | 3.16 | 0.141 | 4% |
| 2 | 6.44 | 0.261 | 4% | 6.41 | 0.261 | 4% | 6.30 | 0.337 | 5% | 6.39 | 0.286 | 4% |
| 3 | 4.22 | 0.127 | 3% | 4.20 | 0.136 | 3% | 4.01 | 0.098 | 2% | 4.16 | 0.121 | 3% |
| 4 | 2.70 | 0.091 | 3% | 2.68 | 0.097 | 4% | 2.67 | 0.081 | 3% | 2.68 | 0.090 | 3% |
| 5 | 0.20 | 0.014 | 7% | 0.19 | 0.011 | 6% | 0.18 | 0.009 | 5% | 0.19 | 0.011 | 6% |
| 6 | 0.24 | 0.032 | 13% | 0.23 | 0.030 | 13% | 0.23 | 0.031 | 13% | 0.24 | 0.025 | 13% |

Table 3
HSV 1 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 | 2.82 | 0.107 | 4% | 2.75 | 0.101 | 3% | 3.05 | 0.141 | 4% | 2.87 | 0.172 | 6% |
| 2 | 5.31 | 0.103 | 2% | 5.07 | 0.237 | 4% | 5.46 | 0.127 | 2% | 5.28 | 0.228 | 4% |
| 3 | 3.47 | 0.063 | 2% | 3.25 | 0.186 | 5% | 3.51 | 0.207 | 6% | 3.41 | 0.196 | 6% |
| 4 | 2.08 | 0.066 | 3% | 2.08 | 0.069 | 3% | 2.12 | 0.056 | 2% | 2.09 | 0.064 | 3% |
| 5 | 0.19 | 0.040 | 2% | 0.22 | 0.020 | 9% | 0.22 | 0.006 | 3% | 0.21 | 0.029 | 14% |
| 6 | 0.17 | 0.013 | 8% | 0.17 | 0.007 | 4% | 0.16 | 0.010 | 6% | 0.16 | 0.011 | 7% |

Table 4
HSV 1 Type Specific IgG Inter Site Precision

| Sample ID | Site 1 | Site 2 | Site 3 | S.D. | C.V. |
|-----------|-----------|-----------|-----------|-------|------|
| | \bar{X} | \bar{X} | \bar{X} | | |
| 1 | 2.79 | 3.16 | 2.87 | 0.195 | 7% |
| 2 | 5.24 | 6.39 | 5.28 | 0.653 | 12% |
| 3 | 3.52 | 4.16 | 3.41 | 0.405 | 11% |
| 4 | 2.20 | 2.68 | 2.09 | 0.314 | 14% |
| 5 | 0.13 | 0.19 | 0.21 | 0.042 | 24% |
| 6 | 0.06 | 0.24 | 0.16 | 0.090 | 59% |

 \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):**

A CDC serum panel was obtained and tested by Trinity Biotech. The results of this testing are presented as a means to convey further information on the performance of this assay with a masked, characterized serum panel. This does not imply an endorsement of the assay by the CDC.

The panel consisted of 58.0% HSV 1 positive and 42.0% HSV 1 negative specimens. The Trinity Biotech HSV 1 Type Specific IgG ELISA demonstrated 96.0% total agreement with the CDC results. Of the results obtained by Trinity Biotech, there was 93.1% agreement with all HSV 1 positive specimens (This includes sera that are positive for HSV 1 only and sera that are positive for both HSV 1 and HSV 2). There was 100.0% agreement with the specimens that were HSV 1 positive only and 100.0% agreement with the specimens that were HSV negative for both.

d. **Type Specificity with HSV 2 Western Blot Positives**

An outside investigator at a Pacific Northwest University assessed the type specificity using HSV 2 Western Blot positive and HSV 1 Western Blot negative sera from the above described populations (n = 56): expectant mothers, sexually active adults, low prevalence persons, and HSV 1 culture positives. Of 56 HSV 2 Western Blot positive and HSV 2 Western Blot negative samples, Trinity ELISA was 54 negative and 2 positive.

Type Specificity with HSV 2 Western Blot Positives (n = 56)

| <i>Characteristic</i> | <i>% (EL/WB)</i> | <i>95% Confidence Interval (CI)</i> |
|--------------------------------------|------------------|-------------------------------------|
| Type-specificity relative to WB | 96.4% (54/56) | 87.7-99.6% |
| Type cross-reactivity relative to WB | 3.6% (2/56) | 0.43-12.3% |

e. **Detection limit:** N/A

f. **Analytical specificity:** Cross-Reactivity with Taxonomically Related Viruses

A study was performed by the manufacturer to determine the cross-reactivity of the Trinity Biotech HSV 1 Type Specific IgG ELISA test with 31 sera containing IgG antibody to taxonomically similar viruses including Cytomegalovirus (CMV), Varicella-Zoster Virus (VZV), and Epstein-Barr Virus (EBV). Of the 31 sera, 5 tested positive for CMV IgG by ELISA, 25 tested positive for VZV IgG by ELISA, and 30 tested positive for EBV IgG by ELISA. All 31 sera were negative by the Trinity Biotech HSV 1 Type Specific IgG ELISA indicating that antibodies to these viruses do not cross-react with the Trinity Biotech HSV 1 Type Specific IgG ELISA. Consequently, because adequate cross-reactivity testing was not performed the sponsor has added a Warning in the Cross-reactivity section of the PI to state, "**Because minimal cross-reactivity performance testing**

| Characteristic | % (TBU ELISA / Alt. ELISA) | 95% Confidence Interval (CI) |
|----------------------------|----------------------------|------------------------------|
| Percent Positive Agreement | 93.88 % (92 / 98) | 87.2 – 97.7% |
| Percent Negative Agreement | 97.06 % (99 / 102) | 91.6 – 99.4 % |
| Percent Agreement | 95.50 % (191 / 200) | 91.6 – 97.9 % |

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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.”

- g. **Assay cut-off:** Because this is a qualitative assay, low level performance testing was done as reproducibility. See above, M.1.a. In addition, 48 HSV Type 1 negative sera were assayed by the HSV 1 IgG Type Specific ELISA test. The mean and standard deviation of the optical density readings for the sera was 0.155 and 0.073 respectively. The positive threshold for the assay was determined by adding the mean and 2.5 standard deviations ($0.155 + 2.5 (0.073) = 0.338$). 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. 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 to Alternate HSV 1 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 1 Type Specific IgG kit and an alternate HSV 1 type specific IgG ELISA test with 200 prospective, unselected, sequentially submitted specimens.

| Prospectively Collected, Sequential Sera | Alternate HSV 1 Type Specific IgG | | |
|--|-----------------------------------|----|---|
| | + | - | E |
| TrinityBiotechCaptia | 92 | 3 | 0 |
| HSV 1 Type Specific | 6 | 99 | 0 |
| | 0 | 0 | 0 |

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

7. 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 1 Western Blot (WB) from a Pacific Northwest university. Of 155 WB positives, Trinity ELISA was 136 positive, 18 negative and 1 equivocal. Of 55 WB negatives, Trinity ELISA was 54 negative and 1 positive.

| Characteristic | % (EL/WB)* | 95% Confidence Interval (CI) |
|----------------------------|------------------|------------------------------|
| % agreement positive to WB | 88.31% (136/154) | 83.2-93.4%‡ |
| % agreement negative to WB | 98.18% (54/55) | 90.3-100.0% |

* Excludes one 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.

‡ 95% CI calculated using the normal approximate method.

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 1 Western Blot (WB) from a Pacific Northwest university. Of 116 WB positives, Trinity ELISA was 102 positive and 14 negative. Of 80 WB negatives, Trinity ELISA was 80 negative.

| Characteristic | % (EL/WB)* | 95% Confidence Interval (CI) |
|----------------------------|------------------|------------------------------|
| % agreement positive to WB | 87.93% (102/116) | 82.0-93.9%‡ |
| % agreement negative to WB | 100.00% (80/80) | 95.5-100.0% |

Excludes two atypical Western Blots.

† 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.

‡ 95% CI calculated using the normal approximate method.

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 1 Western Blot (WB) from a Pacific Northwest university. Of 131 WB negatives, Trinity ELISA was 128 negative, 1 positive and 2 equivocal. Of 53 WB positives, Trinity ELISA was 42 positive, 8 negative and 3 equivocal.

| Characteristic | % (EL/WB)* | 95% Confidence Interval (CI) |
|----------------------------|------------------|------------------------------|
| % agreement positive to WB | 84.00% (42/50) | 70.9-92.8% |
| % agreement negative to WB | 99.22% (128/129) | 95.8-100.0% |

* Excludes five 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.

iv) % Agreement Positive with Culture Positives†

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 1 positive (n = 53). Reference methods included culture (infection) and an HSV 1 Western Blot (WB) (antibody) from a Pacific Northwest university. Of 53 culture positives: 1) Trinity ELISA was 37 positive, 12 negative and 4 equivocal and, 2) WB was 44 positive and 9 negative. Of 44 WB positives: Trinity ELISA was 36 positive, 6 negative, and 2 equivocal.

% Agreement Positive with Culture Positives (n = 53)†

| Characteristic | % (EL/WB or Culture) | 95% Confidence Interval (CI) |
|---------------------------------|----------------------|------------------------------|
| % agreement positive to culture | 75.51% (37/49)* | 61.1-86.7% |
| % agreement positive to WB | 85.71% (36/42)** | 71.5-94.6% |

Excludes four ELISA equivocal.

** Excludes two ELISA equivocal.

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

b. **Clinical specificity:** Specificity testing was performed with only taxonomically related viruses and a limitation added to the product insert.

4. Clinical cut-off: There was only an analytical cutoff determination performed.
5. Expected values/Reference range: Pending receipt of appropriate information.

N. Conclusion:

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