

**510(k) SUBSTANTIAL EQUIVALENCE DETERMINATION  
DECISION SUMMARY**

**A. 510(k) Number:**

K131334

**B. Purpose for Submission:**

Clearance of new diagnostic device

**C. Measurand:**

Human Herpes Simplex Virus Type 1 (HSV-1) specific IgG antibodies to the HSV glycoprotein G1

**D. Type of Test:**

Enzyme linked immunosorbent assay (ELISA)

**E. Applicant:**

Gold Standard Diagnostics

**F. Proprietary and Established Names:**

Herpes Simplex Virus Type 1 IgG ELISA Test

**G. Regulatory Information:**

1. Regulation section: 21 CFR 866.3305, Herpes simplex virus serological assays
2. Classification: Class II
3. Product code: MXJ - Enzyme linked immunosorbent assay, Herpes Simplex Virus, HSV-1
4. Panel: Microbiology (83)

**H. Intended Use:**

1. Intended use(s):

The Herpes Simplex Virus Type 1 IgG ELISA Test Kit is intended for the qualitative detection of IgG antibodies to Herpes Simplex Virus Type 1 in human serum. The test is indicated for sexually active individuals and expectant mothers as an aid for the presumptive diagnosis of HSV-1 infection.

The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The test is not intended for screening of blood and plasma donors. The performance of this assay has not been established for use in a pediatric population, neonates, or immunocompromised patients.

2. Indication(s) for use:

Same as intended use

3. Special conditions for use statement(s):

For prescription use only

4. Special instrument requirements:

Spectrophotometer

**I. Device Description:**

The Gold Standard Diagnostics Herpes Simplex Virus (HSV) Type 1 IgG ELISA Test is an enzyme linked immunosorbent assay for the qualitative detection of IgG antibodies to HSV glycoprotein G1 (gG1) in human serum. The test system consists of recombinant HSV-1 antigen and Horse Radish Peroxidase conjugated goat anti-human IgG (Fc chain specific) to detect IgG class antibodies to HSV-1 in human sera. The kit includes a negative control, positive control, and a cut-off control. The cut-off control is used to determine the validity of the assay and subsequently to determine the result of the sample. Positive and negative controls are provided to determine if the assay is functioning properly. The kit contains 12 x 8 well antigen coated microtiter strips in a frame. The reagents are sufficient for 96 determinations.

**J. Substantial Equivalence Information:**

1. Predicate device name(s):

HerpeSelect<sup>®</sup> 1 and 2 Immunoblot IgG (Focus Diagnostics)

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

K000238

3. Comparison with predicate:

| <b>Similarities</b> |   |  |
|---------------------|---|--|
| <b>Item</b>         | <b>New Device:<br/>Gold Standard Diagnostics,<br/>Herpes Simplex Virus Type 1<br/>IgG ELISA Test Kit</b>  | <b>Predicate:<br/>Focus Diagnostics,<br/>HerpeSelect 1 and 2<br/>Immunoblot IgG</b>  |
| Intended Use        | <p>The Herpes Simplex Virus Type 1 IgG ELISA Test Kit is intended for the qualitative detection of IgG antibodies to Herpes Simplex Virus Type 1 in human serum. The test is indicated for sexually active individuals and expectant mothers as an aid for the presumptive diagnosis of HSV-1 infection.</p> <p>The predictive value of positive or negative results depends on the population's prevalence and the pretest likelihood of HSV-1. The test is not intended for screening of blood, plasma and organ donors. The performance of this assay has not been established for use in a pediatric population, neonates, or immunocompromised patients.</p> | <p>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 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.</p> <p>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.</p> |
| Assay               | Immunoassay   | Immunoassay  |
| Sample Matrix       | Human Serum   | Human Serum  |
| Analyte             | HSV-1 specific human IgG  | HSV-1 and 2 specific human IgG   |
| Antigen Used        | recombinant glycoprotein 1 (gG1)  | recombinant glycoprotein 1 (gG1)   |
| Conjugate           | Goat anti-human IgG   | Goat anti-human IgG  |
| Controls            | Positive Control, Negative Control  | Positive Control, Negative Control   |

| Differences  |  |  |
|--|--|--|
| Item   | New Device   | Predicate  |
| Detection Method   | Colorimetric<br>(Spectrophotometric)   | Visual   |
| Sample volume  | 0.1 mL   | 2 mL   |
| Solid Phase  | Polystyrene 96 well plate<br>(ELISA)   | Nitrocellulose membrane<br>(Western Blot)  |
| Incubation:<br>Time & Temperature                                | 90 Minutes total at 37°C   | 150 Minutes total at RT  |
| Reagents:<br>– Conjugate Label<br>– Substrate<br>– Stop Solution | – horseradish peroxidase<br>(HRP)<br>– TMB*<br>– Acid Mixture                                  | – alkaline phosphatase (AP)<br>– BCIP & NBT**<br>– Deionized water   |
| Result/Unit  | qualitative units determined as<br>$U = (OD_{\text{samples}} / OD_{\text{cut-off}}) \times 10$ | positive/negative;<br>no units reported  |
| Calibrators  | Cut-off Control  | No calibrator; Positive Control<br>serves to determine cut-off   |
| Reading  | Read the color of the wells as<br>optical density  | Visually compare each band on<br>a strip relative to the control<br>bands  |
| Interpretation Criteria  | Negative: < 9<br>Equivocal: 9 to 11<br>Positive: > 11  | Positive: If patient band equal<br>or darker than the positive<br>control band<br>Negative: If band is lighter than<br>the negative control band |

\* TMB: tetramethylbenzidine

\*\* BCIP: bromo-chloro-indolyl-phosphate; NBT: nitroblue-tetrazolium

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

1. Class II Special Controls Guidance Document: Herpes Simplex Virus Types 1 & 2 Serological Assays, September 28, 2010.  
<http://www.fda.gov/MedicalDevices/DeviceRegulationandGuidance/GuidanceDocuments/ucm227411.htm>
2. CLSI EP5: Evaluation of Precision Performance of Clinical Chemistry Devices-Second Edition, Villanova PA
3. CLSI EP7-A2: Interference Testing in Clinical Chemistry; Approved Guideline, 2nd Ed. (2005).

#### L. Test Principle:

The Gold Standard Diagnostics Herpes Simplex Virus (HSV) Type 1 IgG ELISA Test is an enzyme linked immunosorbent assay for the qualitative detection of IgG antibodies to HSV-1

in human serum. The assay requires a total of 90 minutes incubation time. The test uses microtiter wells coated with recombinant gG1 protein of HSV-1. Serum is added to each well and incubated for 30 minutes at 37°C. If antibodies that recognize gG1 protein of HSV-1 are present, they will bind to the antigen in the well. Unbound antibodies are removed by washing the wells three times. A Horse Radish Peroxidase (HRP)-conjugated goat anti-human IgG (conjugate) is then added to each well and incubated for 30 minutes at 37°C. If antibodies against gG1 protein of HSV-1 are present in the patient's serum, the conjugate will bind to the antibody attached to the antigen on the well. The wells are again washed to remove any unbound conjugate. In order to detect the bound conjugate, a substrate containing tetramethylbenzidine (TMB) is added to each well and incubated for 30 minutes at 37°C. If conjugate is present, the HRP will react with the substrate to generate a colored product. After the incubation period, the reaction is stopped with a Stop Solution and the color intensity is measured spectrophotometrically. The kit also includes a Wash Buffer, Diluent, a Negative Control, Positive Control, and a Cut-off Control. The cut-off control is used to determine the validity of the run assay and subsequently to determine the result of the sample. Positive and negative controls are provided to determine if the assay is functioning properly. The kit contains 12 x 8 well antigen coated microtiter strips in a frame. The reagents are sufficient for 96 determinations.

**M. Performance Characteristics (if/when applicable):**

1. Analytical performance:

a. *Precision/Reproducibility:*

**Precision:** To determine precision of the device, a within-laboratory precision study was conducted. Six samples with concentrations that span the dynamic range of the assay were tested in duplicates over twelve days in two runs per day using three lots for a total of 144 results per sample. Each lot was tested for four days. The mean results are summarized in the table below.

| <b>Table 1: Summary of In-House Precision</b> |          |              |    |                   |                    |                    |              |
|---|----------|--------------|----|-------------------|--------------------|--------------------|--------------|
| <b>Sample</b>                                 | <b>N</b> | <b>Units</b> |    | <b>Within-Run</b> | <b>Between-Run</b> | <b>Between-Day</b> | <b>Total</b> |
| High Positive                                 | 48       | 58.452       | SD | 1.544             | 1.261              | 5.644              | 7.652        |
|   |          |              | CV | 2.6%              | 2.5%               | 9.7%               | 13.1%        |
| Moderate Positive                             | 48       | 24.955       | SD | 1.290             | 1.088              | 2.982              | 3.653        |
|   |          |              | CV | 5.2%              | 5.0%               | 11.9%              | 14.6%        |
| Low Positive                                  | 48       | 18.183       | SD | 0.664             | 0.601              | 1.856              | 2.732        |
|   |          |              | CV | 3.7%              | 3.8%               | 10.2%              | 15.0%        |
| Near Cut-off                                  | 48       | 13.691       | SD | 0.601             | 0.501              | 1.388              | 1.871        |
|   |          |              | CV | 4.4%              | 4.2%               | 10.1%              | 13.7%        |
| High Negative                                 | 48       | 6.565        | SD | 0.388             | 0.343              | 0.579              | 0.950        |
|   |          |              | CV | 5.9%              | 6.1%               | 8.8%               | 14.5%        |
| Negative                                      | 48       | 1.899        | SD | 0.153             | 0.133              | 0.256              | 0.342        |
|   |          |              | CV | 8.0%              | 8.0%               | 13.5%              | 18.0%        |

**Reproducibility:** To determine the reproducibility of the assay, six samples (a high positive, a low positive, a near positive cut-off, a near negative cut-off, a high negative, and a negative sample) were tested at three different sites over the course of five days. Two technicians performed one run each per day for a total of two runs per day. A total of 60 measurements per sample were obtained. The mean results of the overall and per site reproducibility are summarized in the tables below:

| Sample           | N  | Mean (Units) |    | Within-Run | Between-Run | Between-Day | Between-Site | Overall Total |
|------------------|----|--------------|----|------------|-------------|-------------|--------------|---------------|
| High Positive    | 60 | 60.909       | SD | 2.487      | 2.535       | 3.749       | 4.952        | 5.788         |
|                  |    |              | CV | 4.1%       | 4.2%        | 6.2%        | 8.1%         | 9.5%          |
| Low Positive     | 60 | 14.754       | SD | 0.775      | 0.758       | 0.957       | 1.369        | 2.156         |
|                  |    |              | CV | 5.2%       | 5.1%        | 6.5%        | 9.3%         | 14.6%         |
| Positive Cut-off | 60 | 11.821       | SD | 0.235      | 0.235       | 0.379       | 0.615        | 0.617         |
|                  |    |              | CV | 2.0%       | 2.0%        | 3.2%        | 5.2%         | 5.2%          |
| Negative Cut-off | 60 | 9.585        | SD | 0.108      | 0.105       | 0.174       | 0.254        | 0.280         |
|                  |    |              | CV | 1.1%       | 1.1%        | 1.8%        | 2.7%         | 2.9%          |
| High Negative    | 60 | 7.508        | SD | 0.194      | 0.195       | 0.447       | 0.760        | 0.821         |
|                  |    |              | CV | 2.6%       | 2.6%        | 6.0%        | 10.1%        | 10.9%         |
| Negative         | 60 | 1.579        | SD | 0.093      | 0.094       | 0.160       | 0.213        | 0.231         |
|                  |    |              | CV | 5.9%       | 6.0%        | 10.1%       | 13.5%        | 14.6%         |

| Sample           | N  | Mean   |    | Within-Run | Between-Run | Between-Day | Overall Total |
|------------------|----|--------|----|------------|-------------|-------------|---------------|
| High Positive    | 20 | 59.224 | SD | 2.569      | 2.643       | 3.370       | 4.360         |
|                  |    |        | CV | 4.3%       | 4.5%        | 5.7%        | 7.4%          |
| Low Positive     | 20 | 13.302 | SD | 0.868      | 0.845       | 0.942       | 1.303         |
|                  |    |        | CV | 6.5%       | 6.3%        | 7.1%        | 9.8%          |
| Positive Cut-off | 20 | 11.759 | SD | 0.133      | 0.138       | 0.291       | 0.585         |
|                  |    |        | CV | 1.1%       | 1.2%        | 2.5%        | 5.0%          |
| Negative Cut-off | 20 | 9.432  | SD | 0.131      | 0.121       | 0.192       | 0.239         |
|                  |    |        | CV | 1.4%       | 1.3%        | 2.0%        | 2.5%          |
| High Negative    | 20 | 7.944  | SD | 0.205      | 0.199       | 0.451       | 0.752         |
|                  |    |        | CV | 2.6%       | 2.5%        | 5.7%        | 9.5%          |
| Negative         | 20 | 1.693  | SD | 0.079      | 0.082       | 0.165       | 0.22          |
|                  |    |        | CV | 4.7%       | 4.8%        | 9.8%        | 12.9%         |

| Table 4: Summary of Reproducibility for Site 2 |    |        |    |            |             |             |               |
|--|----|--------|----|------------|-------------|-------------|---------------|
| Sample   | N  | Mean   |    | Within-Run | Between-Run | Between-Day | Overall Total |
| High Positive                                  | 20 | 58.440 | SD | 2.596      | 2.662       | 4.218       | 5.583         |
|  |    |        | CV | 4.4%       | 4.6%        | 7.2%        | 9.6%          |
| Low Positive                                   | 20 | 13.875 | SD | 0.787      | 0.767       | 0.990       | 1.462         |
|  |    |        | CV | 5.7%       | 5.5%        | 7.1%        | 10.5%         |
| Positive Cut-off                               | 20 | 11.846 | SD | 0.213      | 0.210       | 0.285       | 0.671         |
|  |    |        | CV | 1.8%       | 1.8%        | 2.4%        | 5.7%          |
| Negative Cut-off                               | 20 | 9.712  | SD | 0.102      | 0.104       | 0.193       | 0.249         |
|  |    |        | CV | 1.1%       | 1.1%        | 2.0%        | 2.6%          |
| High Negative                                  | 20 | 7.282  | SD | 0.223      | 0.230       | 0.324       | 0.760         |
|  |    |        | CV | 3.1%       | 3.2%        | 4.5%        | 10.4%         |
| Negative                                       | 20 | 1.473  | SD | 0.10       | 0.10        | 0.15        | 0.20          |
|  |    |        | CV | 6.6%       | 6.6%        | 10.1%       | 13.7%         |

| Table 5: Summary of Reproducibility for Site 3 |    |        |    |            |             |             |               |
|--|----|--------|----|------------|-------------|-------------|---------------|
| Sample   | N  | Mean   |    | Within-Run | Between-Run | Between-Day | Overall Total |
| High Positive                                  | 20 | 65.062 | SD | 2.296      | 2.300       | 3.660       | 4.913         |
|  |    |        | CV | 3.5%       | 3.5%        | 5.6%        | 7.6%          |
| Low Positive                                   | 20 | 17.085 | SD | 0.669      | 0.663       | 0.939       | 1.341         |
|  |    |        | CV | 3.9%       | 3.9%        | 5.5%        | 7.8%          |
| Positive Cut-off                               | 20 | 11.857 | SD | 0.359      | 0.356       | 0.561       | 0.588         |
|  |    |        | CV | 3.0%       | 3.0%        | 4.7%        | 5.0%          |
| Negative Cut-off                               | 20 | 9.610  | SD | 0.090      | 0.091       | 0.138       | 0.275         |
|  |    |        | CV | 0.9%       | 0.9%        | 1.4%        | 2.9%          |
| High Negative                                  | 20 | 7.298  | SD | 0.153      | 0.155       | 0.565       | 0.770         |
|  |    |        | CV | 2.1%       | 2.1%        | 7.7%        | 10.5%         |
| Negative                                       | 20 | 1.571  | SD | 0.10       | 0.10        | 0.17        | 0.22          |
|  |    |        | CV | 6.5%       | 6.6%        | 10.5%       | 13.9%         |

b. *Linearity/assay reportable range:*

Not applicable since this is a qualitative test.

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

Traceability:

There is no standard available for measuring HSV-1 antibody in serum.

Sample Stability:

| <b>Table 6: Recommended Sample Storage for Serum</b> |                |
|--|----------------|
| Stored at room temperature (20-25°C)                 | Up to 8 hours  |
| Stored at 2-8°C                                      | Up to 48 hours |
| Stored at -20°C                                      | >48 hours      |

Reagent Stability:

The shelf life for all unopened kit components has been set to 18 months at 2 to 8°C. The shelf life of final diluted (ready to use) washing solution is given as up to 4 weeks at room temperature. The single components can be used for up to 3 months after the first opening.

Expected values for Controls:

Negative, positive and cut-off controls are provided with every kit. They are supplied as ready to use solutions of human sera with protein-stabilizer and preservative (0.1% ProClin<sup>®</sup>150). Controls are provided with a Quality Control Certificate that indicates the maximum OD-value that may be reached by the negative control and the minimum OD-values that are to be reached for the positive and the cut-off controls. Only if the controls meet the requirements specified on the Quality Control Certificates the assay is valid and results can be calculated.

Units of the cut-off controls are defined as 10 Units. The calculated units of the positive controls should be within the ranges mentioned in the Quality Control Certificate.

d. *Detection limit:*

Not applicable since this is a qualitative test.

e. *Analytical specificity:*

Cross-reactivity:

Potential cross reactivity was evaluated using sera that were tested sero-positive for CMV, EBV, VZV, *Chlamydia trachomatis*, *Treponema pallidum*, Human papilloma virus (HPV), Rubella Virus, *Toxoplasma gondii*, *Candida albicans*, *Neisseria gonorrhoea*, Rheumatoid Factor, ANA, Measles Virus, HSV-2, HIV, and *Bacteroides*. Ten serum samples for each possible cross-reacting antibody were tested in singlicate (except for *Bacteroides*). Sera were obtained from serum brokers who confirmed positivity for the disease and condition using FDA cleared tests. A total of 16 analytes were tested for possible cross reactivity with 151 samples. Samples were tested on the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA

Test as well as on the predicate device. All but 11 samples were found to be nonreactive (negative) in the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA Test. The reactive samples were also reactive on the predicate device, the Focus HerpeSelect 1 Immunoblot IgG, and may therefore represent true positive samples.

Potential cross reactivity with sero-positive specimens against the vector used to express the gG1 recombinant antigen was not assessed.

The results are summarized in the following table:

| <b>Table 7: Summary for Cross-Reactivity Testing</b> |                      |                        |
|--|----------------------|------------------------|
| <b>Positive For</b>                                  | <b>Number Tested</b> | <b>Number Reactive</b> |
| Cytomegalovirus (CMV)                                | 10                   | 0                      |
| Epstein-Barr Virus (EBV)                             | 10                   | 1*                     |
| Varicella-zoster Virus (VZV)                         | 10                   | 1*                     |
| <i>Chlamydia trachomatis</i>                         | 10                   | 0                      |
| <i>Treponema pallidum</i>                            | 10                   | 0                      |
| Human papilloma virus (HPV)                          | 10                   | 0                      |
| Rubella Virus  | 10                   | 0                      |
| <i>Toxoplasma gondii</i>                             | 10                   | 3*                     |
| <i>Candida albicans</i>                              | 10                   | 2*                     |
| <i>Neisseria gonorrhoea</i>                          | 10                   | 0                      |
| Rheumatoid Factor                                    | 10                   | 0                      |
| ANA  | 10                   | 0                      |
| Measles Virus  | 10                   | 0                      |
| HSV-2  | 10                   | 0                      |
| HIV  | 10                   | 4*                     |
| <i>Bacteroides</i>                                   | 1                    | 0                      |

\*Samples were also reactive with the predicate device.

Interference (Endogenous substances):

The effect of potentially interfering endogenous substances on test results with the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA Test was evaluated. High levels of hemoglobin, bilirubin, cholesterol, triglycerides, and albumin were tested in four different positive serum samples: one equivocal, one weak positive, and two moderately positive sera. These samples were then divided into two aliquots, one of which was treated with an interfering substance and tested in triplicate along with the untreated samples. Test concentrations for the potential endogenous interferents were used as recommended by the guideline “Interference Testing in Clinical Chemistry” from the Clinical and Laboratory Standards Institute (CLSI EP7-A2). The following substances did not affect the performance of the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA Test when tested at

the indicated concentration and applying an acceptance criterion of  $\pm 20\%$  relative to untreated samples.

| <b>Table 8: Summary of Testing of Potential Endogenous Interferents</b> |                       |
|---|-----------------------|
| <b>Substance</b>  | <b>Concentration</b>  |
| Hemoglobin  | 2 g/L                 |
| Bilirubin   | 342 $\mu\text{mol/L}$ |
| Cholesterol   | 13 mmol/L             |
| Triglycerides   | 37 mmol/L             |
| Albumin   | 60 g/L                |

*f. Assay cut-off:*

The cut-off was determined by testing 325 sera comprised of negative specimens, positive specimens, and clinically characterized specimens. The samples were from individuals with suspected HSV infection, pregnant women and sexually active adults and were obtained from serum brokers. The cut-off was determined by testing the samples on the Gold Standard Diagnostics HSV assay and on the predicate device. The mean and standard deviation was established for the negative population. A theoretical cut-off was established to maximize the overall percent agreement of the device and the predicate. To encourage repeat testing of samples close to the cut-off, an equivocal range of  $\pm 10\%$  of the cut-off was established.

The placement of the assay's cut-off was validated by comparison studies using receiver operating characteristic (ROC) analysis. The ROC analysis was performed to compare the area under the curve (AUC) of the Gold Standard Diagnostics Herpes Simplex Virus (HSV) Type 1 IgG ELISA Test Kit to that of the Focus Diagnostics HSV 1 ELISA test using the Focus HSV Immunoblot as the standard. A total of 254 sera were tested in this validation study on all three assays. The AUC of the Gold Standard Diagnostics Herpes Simplex Virus (HSV) Type 1 IgG ELISA Test Kit is similar to the AUC of the Focus Diagnostics HSV Immunoblot. The observed cut-offs of both assays are similar.

Based upon the results of this testing, the manufacturer established the following guidelines for interpretation of patient samples:

| <b>Table 9: Result Interpretation</b> |               |
|---------------------------------------|---------------|
| <b>Units</b>                          | <b>Result</b> |
| < 9.0                                 | negative      |
| 9.0 – 11.0                            | equivocal     |
| > 11.0                                | positive      |

1. If the measured value is above the defined equivocal range, the result is considered to be positive.
2. If the measured value is within the equivocal range, the sample should be retested. If the result remains equivocal on the retest, the patient should be considered suspect for infection.

3. If the measured value is below the defined equivocal range, no measurable antigen specific antibodies are present in the samples. The samples are considered to be negative.

The cut-off as established above was then applied to the prospectively collected samples tested in the method comparison study (described in item 2 below) and validated.

2. Comparison studies:

a. *Method comparison with predicate device:*

The method comparison was based on the results from the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA Test compared to the results obtained from the FDA cleared predicate, the HerpeSelect<sup>®</sup> 1 and 2 Immunoblot IgG from Focus Diagnostics. The testing description and data are listed in the Clinical Study section below (Section 3).

b. *Matrix comparison:*

Not applicable because only serum is claimed.

3. Clinical studies:

The following table provides a summary of all clinical samples that were tested in the clinical study and the testing sites at which they were tested.

| <b>Table 10: Samples tested at the clinical sites</b> |                                       |            |            |                         |
|---|---------------------------------------|------------|------------|-------------------------|
| Populations   | Number of samples tested at each site |            |            | Total number of samples |
|   | Site 1                                | Site 2     | Site 3     |                         |
| Sexually active adults                                | 222                                   | 195        | 101        | 518                     |
| Pregnant women  | 114                                   | 71         | 0          | 185                     |
| Low prevalence population                             | 100                                   | 0          | 0          | 100                     |
| CDC seroconversion panel                              | 0                                     | 0          | 100        | 100                     |
| <b>Total</b>  | <b>436</b>                            | <b>266</b> | <b>201</b> | <b>903</b>              |

**Performance in the Intended Use Populations:**

The performance of the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA assay was determined by conducting a correlation study with clinical samples that were tested at three different sites. A total of 703 samples was used that were representative of the intended use population, sexually active adults and pregnant women. Of this total, 185 samples were from pregnant women and 518 samples were from sexually active adults. The samples were prospectively collected, left-over, de-identified specimens that had been submitted for HSV testing. Specimens were stored up to 48h at 4°C before testing. Those samples that were shipped to the internal testing site at Gold Standard Diagnostics (101 samples from sexually active adults) were frozen at -20°C. All samples were tested on the Gold Standard Herpes Simplex Virus Type 1 IgG ELISA assay and the predicate device. The combined results from all three sites are summarized in Tables 11 and 12 according to the intended use population tested. Equivocal results were treated as the worst case results (disagreement) for calculation of sensitivity and specificity; confidence intervals (C.I.) were calculated using the exact method.

| <b>Table 11</b>  |           |                            |          |
|--|-----------|----------------------------|----------|
| <b>Pregnant Women</b>                                    |           | <b>Predicate Device</b>    |          |
|  |           | Positive                   | Negative |
| <b>Gold Standard<br/>Diagnostics HSV-1<br/>IgG ELISA</b> | Positive  | 96                         | 1        |
|  | Equivocal | 1                          | 2        |
|  | Negative  | 3                          | 82       |
| Sensitivity = 96.0% (96/100)                             |           | (95% C.I. = 90.1% - 98.9%) |          |
| Specificity = 96.5% (82/85)                              |           | (95% C.I. = 90.0% - 99.3%) |          |

| <b>Table 12</b>  |           |                            |          |
|--|-----------|----------------------------|----------|
| <b>Sexually Active Adults</b>                            |           | <b>Predicate Device</b>    |          |
|  |           | Positive                   | Negative |
| <b>Gold Standard<br/>Diagnostics HSV-1<br/>IgG ELISA</b> | Positive  | 275                        | 8        |
|  | Equivocal | 3                          | 2        |
|  | Negative  | 20                         | 210      |
| Sensitivity = 92.3% (275/298)                            |           | (95% C.I. = 88.6% - 95.0%) |          |
| Specificity = 95.5% (210/220)                            |           | (95% C.I. = 91.8% - 97.8%) |          |

**Performance in a Low Prevalence Population:**

A total of 100 samples were requested to be collected from 16-19 year olds in a non-STD setting. The samples of this low prevalence population were tested at only one of the testing sites. They were prospectively collected, left-over, de-identified specimens. Specimens were stored up to 48h at 4°C before testing. Calculation of sensitivity and specificity with their respective confidence intervals was performed as described for the intended use populations above.

| <b>Table 13</b>  |           |                         |          |
|--|-----------|-------------------------|----------|
| <b>Low Prevalence</b>                                    |           | <b>Predicate Device</b> |          |
|  |           | Positive                | Negative |
| <b>Gold Standard<br/>Diagnostics HSV-1<br/>IgG ELISA</b> | Positive  | 15                      | 3        |
|  | Equivocal | 0                       | 3        |
|  | Negative  | 1                       | 78       |

Sensitivity = 93.8% (15/16) (95% C.I. = 69.7% - 99.8%)

Specificity = 92.9% (78/84) (95% C.I. = 85.1% - 97.3%)

**Performance with a CDC Seroconversion Panel:**

A seroconversion panel consisting of well-characterized HSV-1 serum samples was obtained from the CDC and tested on the Gold Standard Herpes Simplex Virus Type 1 IgG ELISA assay. The seroconversion panel consisted of archived (frozen) samples that were tested at only the internal testing site, Gold Standard Diagnostics. The panel consisted of 50 HSV-1 IgG positive samples and 50 HSV-1 IgG negative samples. Note that the use of the CDC panel does not imply endorsement of the assay by the CDC. The results are summarized in Table 14. Calculation of sensitivity and specificity with their respective confidence intervals was performed as described for the intended use populations above.

| <b>Table 14</b>  |           |            |          |
|--|-----------|------------|----------|
| <b>CDC Panel</b>   |           | <b>CDC</b> |          |
|  |           | Positive   | Negative |
| <b>Gold Standard<br/>Diagnostics HSV-1<br/>IgG ELISA</b> | Positive  | 45         | 3        |
|  | Equivocal | 0          | 0        |
|  | Negative  | 1          | 51       |

Sensitivity = 97.8% (45/46) (95% C.I. = 88.5% - 99.9%)

Specificity = 94.4% (51/54) (95% C.I. = 84.6% - 98.8%)

4. Clinical cut-off:

N/A

5. Expected values/Reference range:

The prevalence of HSV-1 IgG antibodies in the intended use populations of pregnant women and sexually active adults was assessed internally and externally at two sites. The assessment was based on the performance of the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA assay with the samples tested in the concordance study. A total of 703 sera were tested. In the population of pregnant women (n=185), the observed prevalence with the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA assay was 53.5% (99/185). With the sexually active individuals (n=518) the observed prevalence with the Gold Standard Diagnostics Herpes Simplex Virus Type 1

IgG ELISA assay was 55% (285/518).

The following two tables (Table 15 and Table 16) summarize the prevalence observed when the investigational device was tested on the two populations: sexually active individuals and pregnant women). Calculation was based on the results that count equivocal samples to the disadvantage of the new device (worst case results).

| Age (years)   | Pos       | Equ      | Neg       | Total      | Prevalence   |
|---------------|-----------|----------|-----------|------------|--------------|
| 16-19         | 7         | 0        | 11        | 18         | <b>38.9%</b> |
| 20-24         | 20        | 0        | 11        | 31         | <b>64.5%</b> |
| 25-29         | 23        | 0        | 18        | 41         | <b>56.1%</b> |
| 30-34         | 26        | 2        | 28        | 56         | <b>46.4%</b> |
| 35-39         | 16        | 1        | 14        | 31         | <b>51.6%</b> |
| >40           | 5         | 0        | 3         | 8          | <b>62.5%</b> |
| <b>Totals</b> | <b>97</b> | <b>3</b> | <b>85</b> | <b>185</b> | <b>53.5%</b> |

| Age (years)   | Pos        | Equ      | Neg        | Total      | Prevalence   |
|---------------|------------|----------|------------|------------|--------------|
| 16-19         | 22         | 0        | 26         | 48         | <b>45.8%</b> |
| 20-24         | 27         | 1        | 50         | 78         | <b>34.6%</b> |
| 25-29         | 55         | 1        | 63         | 119        | <b>46.2%</b> |
| 30-34         | 58         | 1        | 30         | 89         | <b>65.2%</b> |
| 35-39         | 28         | 1        | 22         | 51         | <b>54.9%</b> |
| >40           | 93         | 1        | 39         | 133        | <b>69.9%</b> |
| <b>Totals</b> | <b>283</b> | <b>5</b> | <b>230</b> | <b>518</b> | <b>55%</b>   |

The hypothetical predictive values for the two populations are shown in the table below. The calculations are based on the Gold Standard Diagnostics Herpes Simplex Virus Type 1 IgG ELISA assay.

| Prevalence | Sexually Active Adults |       | Pregnant Women |       |
|------------|------------------------|-------|----------------|-------|
|            | PPV                    | NPV   | PPV            | NPV   |
| 50%        | 95.4%                  | 92.5% | 96.5%          | 96.0% |
| 40%        | 93.2%                  | 94.9% | 94.8%          | 97.3% |
| 30%        | 89.8%                  | 96.7% | 92.2%          | 98.3% |
| 25%        | 87.2%                  | 97.4% | 90.1%          | 98.6% |
| 20%        | 83.7%                  | 98.0% | 87.3%          | 99.0% |
| 15%        | 78.4%                  | 98.6% | 82.9%          | 99.3% |
| 10%        | 69.5%                  | 99.1% | 75.3%          | 99.5% |
| 5%         | 51.9%                  | 99.6% | 59.1%          | 99.8% |

**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.