## 2.0 510(k) SUMMARY – DonorScreen-HLA® Class I and Class II

| | | |
|---|---|---|
| **I.** | **Owner/Manufacturer:** | Immucor GTI Diagnostics, Inc. |
| | **Address**: | 20925 Crossroads Circle, Waukesha, WI 53186, USA |
| | **Phone**: | 262.754.1009 |
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| | **Name of Submitter**: | Julie Ewend [JEwend@immucor.com] |
| | **Date Prepared:** | February 7, 2018 |
| **II.** | **Device Trade Name:** | DonorScreen-HLA® Class I and Class II |
| | **Common Name:** | Test, HLA |
| | **Classification Name:** | Test, Qualitative, For HLA, Non-Diagnostic |
| | **Division:** | CBER |
| | **Review Panel:** | Hematology |
| | **Product Code:** | MZI |
| | **Classification:** | Unclassified |
| | **Submission Type:** | 510(k) |
| | **Device Class:** | 2 |

### III. Name of Device for Claiming Equivalence

DonorScreen-HLA® Class I and Class II (BK070045)

### IV. Description of Device

DonorScreen-HLA® Class I and Class II assay is a qualitative Enzyme Linked Immunosorbent Assay (ELISA) designed to detect anti-HLA class I and class II antibodies in human serum or plasma of blood donors.

The assay is performed on the STRATEC BIOMEDICAL AG® GEMINI (GEMINI) instrument. The instrument is an automated EIA microtiter plate analyzer. The reagents required to perform the assay on the instrument are provided in the DonorScreen-HLA® Class I and Class II kit.

The assay is performed on either full plates of microwell strips to perform 176 tests maximum per kit or a reduced number of microwell strips may be run when testing volume is not maximized. When full plates are not run, reagents within the kit do not support the minimized configuration. The DonorScreen-HLA® Class I and Class II Additional Reagents kit is available to support use of the assay when testing less than the maximum number of samples is performed. The Additional Reagents packaging was not available at time of first release to the market. Labeling is being provided in this submission to describe the composition of the packaging.

Human Leukocyte Antigens (HLAs) are highly polymorphic glycoproteins. HLA antibodies can be acquired through alloimmunization of pregnancy, transfusions, or previous transplantation. In general alloimmunization leads to the production of HLA antibodies in approximately 33% of exposed individuals. The formation of these antibodies in a transfusion or transplant recipient can result in the immune destruction of transfused platelets or the transplanted organ. The presence of pre-existing HLA antibodies in blood donors has also been implicated in Transfusion-Related Acute Lung Injury (TRALI) and TRALI-like transfusion reactions in the recipients of blood products from the donors. However, in 10-15% of TRALI reactions no antibodies are found in the donor(s) and in 45-60% of TRALI reactions neutrophil specific antibodies are found in the donor(s).

The DonorScreen product was designed to meet a need which was initiated by the American Association of Blood Banks (AABB) during 2007 and 2008. The recommendations were to reduce the incidence of TRALI by minimizing the incidence of high plasma-volume components from donors known to be leukocyte-alloimmunized or at increased risk of leukocyte alloimmunization. This measure could include the testing of blood donors that fall into this category for anti-HLA class I and class II antibodies.

The DonorScreen product has met this need in the market since 510(k) clearance of the product in July 2008. The instrument platform included with the assay at the time of clearance was the Bio-Rad QuickStep® instrument. At this time, a newer instrument is available in the market which has been validated to be used with the DonorScreen assay. This instrument performs the same functions that the QuickStep instrument performs. It is also manufactured by the same OEM, Stratec Biomedical AG.

DonorScreen-HLA® Class I and Class II has been specifically designed to be used on the GEMINI automated ELISA instrument. Result evaluation is carried out on the instrument. Minimal reagent preparation by the user is required.

Test Procedure

Donor serum or plasma is diluted with Specimen Diluent and added to microwells coated with affinity purified HLA class I or HLA class II glycoproteins allowing antibody, if present, to bind. Unbound antibodies are then washed away. An alkaline phosphatase labeled anti-human globulin (Anti-IgG) is added to the wells and incubated. The unbound Anti-IgG is washed away and the substrate PNPP (p-nitrophenyl phosphate) is added. After incubation at ambient temperature, the reaction is stopped by a Stopping Solution. The optical density of the color that develops is measured in a spectrophotometer at 405 nm with a reference wavelength of 492 nm. All of the above steps are carried out by the GEMINI automated ELISA instrument.

Quality control of the DonorScreen-HLA® Class I and Class II assay is built into the assay

by inclusion of Positive and Negative Serum Controls. Two replicates of the Positive Serum Control and four replicates of the Negative Serum Control are automatically processed with each assay. The Quality Control values are pre-programmed into the DonorScreen-HLA assay file. If any one of these values is not met, the O.D. values for each sample will still be printed but the Qualitative Result will not appear on the electronic or printed report. An error code of FAILED will appear on page 1 of the report and computer screen.

## V. Intended Use

DonorScreen-HLA® Class I and Class II assay is a qualitative Enzyme Linked Immunosorbent Assay (ELISA) for use on the STRATEC BIOMEDICAL AG® GEMINI instrument. DonorScreen-HLA® Class I and Class II assay ELISA is designed to detect anti-HLA class I and class II antibodies in human serum or plasma of blood donors.

For *In Vitro* Diagnostic (IVD) Use.

## VI. Substantial Equivalence

Within this submission, the studies performed as described previously to the agency through a Pre-Sub Meeting Request (BQ160055) to meet migration requirements per FDA Guidance: Assay Migration Studies for In Vitro Diagnostic Devices (April 25, 2013) and a reagent substitution to the DonorScreen-HLA® Class I and Class II assay are being provided. The Instructions for Use for the DonorScreen-HLA® Class I and Class II assay is provided for the performance of the assay on the GEMINI instrument system. A summary of changes within the IFU from time of clearance to date are also being described. The company name has changed from Genetic Testing Institute, Inc. to Immucor GTI Diagnostics, Inc. These changes are being described in subsections 1-5 of this section.

### 1. Summary of Similarities and Differences between the Bio-Rad QuickStep® and the STRATEC BIOMEDICAL AG® GEMINI

Similarities between the Bio-Rad QuickStep® (QuickStep) and STRATEC BIOMEDICAL AG® GEMINI (GEMINI) instruments:

1. Both systems are made by the same OEM manufacturer.
2. The GEMINI instrument contains multiple systems which are substantially similar to the QuickStep instrument such as the pipetting, washer, and reader systems.
3. The User Software for the GEMINI instrument includes the same features as the QuickStep.

4. The QuickStep and GEMINI perform the same function as microplate processors. Both systems perform sample dilution, sample and reagent dispensing, incubations, wash processes, and plate transport. In addition, both systems collect data using an onboard spectrophotometer and process the data to provide an analysis and results report.

Differences between the QuickStep and GEMINI instruments:

1. The QuickStep can process 4 plates while the GEMINI can process 2 – 3 plates at the same time.
2. The GEMINI software is qualified and provided with a current version of the Windows operating system.
3. The GEMINI software has additional features which were not available on the QuickStep including drift compensation.
4. The GEMINI instrument has a wider equipment operating temperature range than the QuickStep.
5. The GEMINI system has a smaller footprint than the QuickStep system.
6. The GEMINI has a Back to Source feature that is used to return unused reagent to the source bottles for Conjugate, Substrate and Stopping Solution.

Table 1 provides a comparison of the QuickStep instrument and the GEMINI instrument.

The table below has been updated compared to the Table originally provided to the Agency at the time of the Pre-Submission meeting correspondence (rev 7, June 2016). GEMINI equipment specifications listed in the GEMINI IFU rev 10 were inforce during the applicable studies performed and described in this submission.

**Table 1: Equipment Manufacturer Specifications**

| | **Bio-Rad QuickStep®** | **STRATEC BIOMEDICAL AG® GEMINI** |
|---|---|---|
| Maximum number of plates | 4 | 2 – 3 |
| Instrument Dimensions | 114 cm x 156 cm x 100 cm<br>*Includes space needed for open reagent drawer and waste tip bag* | 97 cm x 66 cm x 75 cm |
| Operating System | Microsoft Windows 95/2000 | Microsoft Windows 7 |
| Instrument software | 1.30.1 | 2.0.6 (2.06) |
| Voltage requirement | 110 V – 230 V ±10% | 110 V – 240 V ±10% |
| Frequency Range | 50 Hz – 60 Hz | 50 Hz – 60 Hz |

| | **Bio-Rad QuickStep®** | **STRATEC BIOMEDICAL AG® GEMINI** |
|---|---|---|
| Acceptable Operating Altitude | Up to 2,000 m above sea level | Up to 2,000 m above sea level |
| Operating Temperature | 15 – 25°C | 15 – 30°C |
| Humidity | Up to 80% relative humidity up to 31°C | 30 – 80% non-condensing humidity |
| Sample & reagent barcode reader | Yes | Yes |
| Photometric Reader Dynamic Range | Up to 3.0 OD | Up to 3.0 OD |
| Photometric Reader Spectral Range | 400 – 700 nm | 400 – 700 nm |
| Photometric Reader Precision | N/A | 1% CV at 1.0 OD |
| Photometric Reader Accuracy | ± 0.005 or 2.5% (whichever is greater) | ± 0.005 or 2.5% (whichever is greater) |
| Photometric Reader Linearity | 0 – 2.000 OD ± 1% | 0 – 2.0 OD ± 1% |
| Pipettor System pipette volumes | Min: 10 – 300 uL using 300uL tip<br>Max: 301 – 1,000 uL using 1,100uL tip | Min: 10 – 300 uL using 300uL tip<br>Max: 301 – 1,000 uL using 1,100uL tip |
| Pipettor System Accuracy | < -15% at 25 uL<br>< -5% at 100 uL | N/A |
| Pipettor System Precision | < 5% CV at 25 uL<br><2.5% CV at 100 uL | Single Dispense<br>< 3% CV at 20 uL<br>< 3% CV at 100 uL<br><br>Multi Dispense<br>< 10% CV at 16 x 20 uL<br>< 3% CV at 8 x 100 uL |
| Pipettor System Features | Liquid level detection, tip detection, mixing, multi-dispensing | Pipetting pressure monitoring (optional), capacitive liquid level detection, tip detection, mixing, multiple dilution steps, archiving. |
| Incubation Temperature Range | 7°C above room temperature to 50°C | Up to 45°C |
| Incubation Temperature Uniformity | - 1.5°C to + 1°C | ± 1.5°C |
| Washer Head | 1 row of dispense and aspirate needle pairs | 1 row of dispense and aspirate needle pairs |
| Washer Precision | 10% CV at 300 uL | 10% CV at 300 uL |

| | Bio-Rad QuickStep® | STRATEC BIOMEDICAL AG® GEMINI |
|---|---|---|
| Washer Residual Volume | < 2.5 uL in U-bottom (mean)<br>< 4 uL in flat bottom (mean) | < 2.5 uL in U-bottom (mean)<br>< 4 uL in flat bottom (mean) |
| Washer Modes | Sweep mode, soak, top & bottom wash, variable pump speed | Sweep mode, soak, top & bottom wash, variable pump speed |

2. **Stopping Solution Composition Change-Sodium Hydroxide to EDTA**

   The Stopping Solution composition was changed from a 3M Sodium Hydroxide solution to a non-hazardous EDTA based solution. The change was validated prior to implementation in the kit. Information to support this change is being provided in Section 15, Analytical Performance Studies.

3. **Additional changes to Instructions for Use (IFU) post original Clearance**

   Changes were made to the Instructions for Use for the DonorScreen-HLA® Class I and Class II product post original 510(k) clearance. Assessments for the changes identified they were not significant changes requiring a new 510(k) submission.

   Expanded description of these changes is provided in Section 13.0, Proposed Labeling or Section 15.0, Analytical Performance Studies.

   The types of changes are as follows:

   Use of symbols instead of wording for temperature and IVD, name change of QuickStep manufacturer, Microwells referred to as Microwell Strips, added numerical reagent inventory management number, change to Stopping Solution composition and alpha code, removal of Caution related to Sodium Hydroxide Stopping Solution, pipette size not used removed from additional materials section, new NOTE referencing acceptable tube sizes for samples and sample volume needed, listed Assay File identifier/name, added in-use stability information for two reagents, wording revision for clarity for “Qualitative Result”, correction of typographical errors, addition of hazard codes table related to Safety Data Sheet.

4. **Company name change**

   Genetic Testing Institute Inc. was purchased by Gen-Probe Inc. in December 2010. The company name was changed to Gen-Probe GTI Diagnostics, Inc.

Purchase of Gen-Probe GTI Diagnostics Inc. by Immucor, Inc. occurred in March 2013. The company name was subsequently changed to Immucor GTI Diagnostics, Inc.

**5. DonorScreen-HLA® Class I and Class II assay file for BioRad QuickStep® Instrument**

*During interactive review with the Agency, this section has been determined to be not applicable to the subject of this 510(k) submission, BK180181, DonorScreen-HLA Class I and Class II for use on the STRATEC BIOMEDICAL AG GEMINI Instrument.*

The characters visible in the sample field in the report were expanded to allow additional sample identification. This was in response to a customer inquiry. The change occurred in January 2016. A description of the modification is included in Section 15.0, Analytical Performance Studies.

## VII. Support of Substantial Equivalence with Performance Data

In correlation to Section VI, above, this section is being organized as two sections:

1. Migration studies Bio-Rad QuickStep® versus STRATEC BIOMEDICAL AG® GEMINI
2. Stopping Solution Change 3M NaOH to Non-hazardous EDTA
3. Additional Studies to Support Assay Performance

Study summaries are provided below. Details of these studies as well as additional Analytical Test data can be found in Sections 15.0, Analytical Performance Studies and 17.0, Clinical Performance Testing.

**1. Migration studies Bio-Rad QuickStep® versus STRATEC BIOMEDICAL AG® GEMINI**

Analytical and clinical performance studies were conducted to support addition of the GEMINI instrument for use with the DonorScreen-HLA assay.

The following studies were described in the Pre-Sub Meeting request. Further direction was not provided confirming that the studies described below would be an acceptable method to show use of the DonorScreen-HLA® Class I and Class II assay on the QuickStep and GEMINI instruments was equivalent. The studies were performed at 2 external clinical sites and as internal studies as defined in

the table below.

| Study/Action | Testing on GEMINI | Testing on QuickStep |
|---|---|---|
| **A. Comparison Study**<br>318 Samples to be tested comprised of either HLA Class I or HLA Class II specific samples or samples which contained both HLA Class I and HLA Class II<br>• 231 HLA Class I<br>• 200 HLA Class II | 2 External Clinical Sites<br>1 Internal Site | 1 Internal Site<br>(2 QuickStep instruments to be used) |
| **B. Reproducibility Study**<br>• 2 true negative samples<br>• 2 high negative samples<br>• 2 low positive samples<br>• 2 moderate positive samples | 1 Internal Site<br>2 External Clinical Sites | 1 Internal Site<br>2 External Clinical Sites |
| **C. Precision Study**<br>• 2 true negative samples<br>• 2 high negative samples<br>• 2 low positive samples<br>• 2 moderate positive samples | 1 Internal Site | 1 Internal Site |
| **D. Lot to Lot Reproducibility**<br>• 2 true negative samples<br>• 2 high negative samples<br>• 2 low positive samples<br>• 2 moderate positive samples | 1 Internal Site | 1 Internal Site |
| **E. Performance at Low Positive Levels**<br>5 low samples and the Negative Control | 1 Internal Site | 1 Internal Site |
| **F. False Positivity Rate** | 1 Internal Site | 1 Internal Site |
| **G. Plasma vs. Serum Comparison**<br>At least 40 paired serum and EDTA plasma samples | 1 Internal Site | 1 Internal Site |

**QuickStep versus GEMINI Study Summaries**

A. Method Comparison Study

The DonorScreen-HLA® Class I and Class II assay was migrated from the QuickStep instrument to the GEMINI instrument. A study was conducted in which the DonorScreen-

HLA® Class I and Class II assay results on the GEMINI system at three (3) sites were compared to results on the existing QuickStep system. Results were analyzed to demonstrate performance on the GEMINI system comparable to the QuickStep.

The Clinical field trial protocol was executed at two external sites and one internal site. The internal site performed testing on one GEMINI and two QuickStep instruments. The external sites performed testing on the Gemini only. One lot of the DonorScreen-HLA® Class I and Class II assay was provided for this study.

Sample panels for HLA class I (231 samples) and HLA class II (200 samples) were assembled. Samples were serum collected from primarily multiparous female donors with a smaller number from nulliparous female and non-transfused male donors.

The results of the study were analyzed for concordance using 2x2 tables. The following tables show the composite results from those studies by HLA class.

*HLA Class I – 1,386 results from 231 samples*

<table>
<tr><td colspan="2" rowspan="2">1386 Results</td><td colspan="2">All QuickStep Instruments (n=2)</td><td>% Agreement</td><td>96.5%</td></tr>
<tr><td>Positive</td><td>Negative</td><td>Concordance (95% Lower CI)</td><td>93.9%*</td></tr>
<tr><td rowspan="4">All GEMINI Instruments (n=3)</td><td rowspan="2">Positive</td><td rowspan="2">677</td><td rowspan="2">41</td><td>PPA (Point Estimate)</td><td>99.0%</td></tr>
<tr><td>PPA (95% Lower CI)</td><td>96.7%*</td></tr>
<tr><td rowspan="2">Negative</td><td rowspan="2">7</td><td rowspan="2">661</td><td>NPA (Point Estimate)</td><td>94.2%</td></tr>
<tr><td>NPA (95% Lower CI)</td><td>89.6%*</td></tr>
</table>

*Overall Lower confidence calculated using SAS PROC SURVEYSELECT

*HLA Class II – 1,200 results from 200 samples*

| **1200 Results** | | **All QuickStep Instruments (n=2)** | | % Agreement | 97.9% |
|---|---|---|---|---|---|
| | | Positive | Negative | Concordance (95% Lower CI) | 95.5%* |
| **All GEMINI Instruments (n=3)** | Positive | 587 | 15 | PPA (Point Estimate) | 98.3% |
| | | | | PPA (95% Lower CI) | 95.3%* |
| | Negative | 10 | 588 | NPA (Point Estimate) | 97.5% |
| | | | | NPA (95% Lower CI) | 94.1%* |

*Overall Lower confidence calculated using SAS PROC SURVEYSELECT

B. Reproducibility Study

Reproducibility studies were performed for DonorScreen-HLA® Class I and HLA Class II assays at all three sites to demonstrate the reproducibility of the results obtained with the GEMINI and the QuickStep.

One panel of 24 samples (triplicates of eight unique samples) was provided to each site. Each site provided two operators to participate in the study and conducted testing consisting of two runs per day for five non-consecutive days.

The results were compared between QuickStep and GEMINI instrument, each QuickStep Instrument and between the QuickStep instruments and each GEMINI Instrument. For the reproducibility study, the GEMINI and Quickstep demonstrated comparable and strong agreement (>97%) for PPA and NPA for both HLA-Class I and HLA-Class II.

C. Precision Study

The repeatability and total imprecision of the DonorScreen-HLA® Class I and Class II assay was determined. Eight (8) samples of varying antibody concentrations were tested in the DonorScreen-HLA® Class I and II assay. Samples included negative samples as well

as positive samples representing low and moderate reactivity. Each assay included two (2) replicates of each sample per assay run. Testing was performed by a single operator on 12 days with 2 assay runs per day, for a total of 24 assay runs. To obtain the imprecision of the OD values, the data were analyzed according to CLSI Document EP-05-A3.

*HLA Class I*

| Sample ID | Expected Result | Average OD | Repeatability | | Between Run | | Between Day | | Total Within-lab | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 1-02 | Negative | 0.110 | 0.007 | 6.4 | 0.003 | 2.7 | 0.004 | 3.6 | 0.008 | 7.3 |
| 1-03 | Negative | 0.114 | 0.005 | 4.4 | 0.006 | 5.3 | 0.004 | 3.5 | 0.009 | 7.9 |
| 1-08 | Negative | 0.096 | 0.008 | 8.3 | 0.003 | 3.1 | 0.006 | 6.3 | 0.010 | 10.4 |
| U-01 | Negative | 0.115 | 0.007 | 6.1 | 0.006 | 5.2 | 0.004 | 3.5 | 0.010 | 8.7 |
| U-06 | Positive | 0.582 | 0.027 | 4.6 | 0.017 | 2.9 | 0.015 | 2.6 | 0.035 | 6.0 |
| 1-05 | Positive | 0.736 | 0.027 | 3.7 | 0.028 | 3.8 | 0.014 | 1.9 | 0.041 | 5.6 |
| 1-07 | Positive | 0.738 | 0.025 | 3.4 | 0.021 | 2.8 | 0.012 | 1.6 | 0.035 | 4.7 |
| U-04 | Positive | 0.967 | 0.064 | 6.6 | 0.024 | 2.5 | 0.033 | 3.4 | 0.076 | 7.9 |

*HLA Class II*

| Sample ID | Expected Result | Average OD | Repeatability | | Between Run | | Between Day | | Total Within-lab | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | SD | %CV | SD | %CV | SD | %CV | SD | %CV |
| 2-11 | Negative | 0.280 | 0.010 | 3.6 | 0.012 | 4.3 | 0.015 | 5.4 | 0.022 | 7.9 |
| 2-12 | Negative | 0.251 | 0.014 | 5.6 | 0.011 | 4.4 | 0.016 | 6.4 | 0.024 | 9.6 |
| 2-13 | Negative | 0.168 | 0.008 | 4.8 | 0.009 | 5.4 | 0.010 | 6.0 | 0.016 | 9.5 |
| 2-14 | Negative | 0.194 | 0.010 | 5.2 | 0.011 | 5.7 | 0.010 | 5.2 | 0.018 | 9.3 |
| 2-15 | Positive | 0.663 | 0.023 | 3.5 | 0.031 | 4.7 | 0.014 | 2.1 | 0.041 | 6.2 |
| U-06 | Positive | 0.711 | 0.019 | 2.7 | 0.029 | 4.1 | 0.014 | 2.0 | 0.037 | 5.2 |
| U-01 | Positive | 1.523 | 0.041 | 2.7 | 0.059 | 3.9 | 0.030 | 2.0 | 0.078 | 5.1 |
| U-04 | Positive | 0.833 | 0.030 | 3.6 | 0.032 | 3.8 | 0.031 | 3.7 | 0.053 | 6.4 |

The target acceptance criteria was met (100% agreement in positive and negative results was obtained with the new GEMINI system): True Negative: 100% negative results; High Negative: ≥ 95% negative results; Low Positive: ≥ 95% positive results; and Moderate Positive: 100% positive results.

The %CV values observed with the new GEMINI system were consistent across the range of OD values tested. Additionally, the ratio of standard deviations (GEMINI SD/QuickStep SD) was evaluated for the repeatability and the total within-lab precision. The data indicates that the DonorScreen-HLA assay tested with the GEMINI system demonstrated improved repeatability and improved total-within lab precision compared to the testing with the QuickStep system.

D. Lot to Lot Reproducibility

Reproducibility between lots of DonorScreen-HLA® Class I and Class II was performed on the new GEMINI system in comparison to the results on the old QuickStep system.

Eight samples were tested with the HLA Class I assay and eight samples were tested with the HLA Class II assay. For both the HLA Class I testing and the HLA Class II testing, there were 4 samples with HLA Class I reactivity and 4 samples with HLA Class II reactivity with expected low to moderate positive OD values. There were also 4 samples negative for HLA Class I reactivity and 4 samples negative for HLA Class II reactivity with expected true negative to high negative reactivity. The study was conducted for 5 days of testing, with 2 runs per day, and 3 replicates of each sample per run. The 5 days were not required to be consecutive. It was also not required that both systems be tested on the same day. All testing was conducted by two operators on both systems (one GEMINI system and one QuickStep system). The study design followed the recommendations described in the Assay Migration Guidance document for the reproducibility study and was proposed in the FDA pre-sub correspondence. The Lot to Lot Reproducibility study data was analyzed for both qualitative (positive, negative) results agreement and for quantitative (OD) output. The results from each system (GEMINI and Quickstep) and each lot were analyzed separately.

Overall, the average OD values obtained were consistent between lots compared within a system. The **qualitative** results of the HLA Class I and Class II assays met the target acceptance criteria as stated below:

Target
True Negative: 100% negative results
High Negative: ≥ 95% negative results
Low Positive: ≥ 95% positive results
Moderate Positive: 100% positive results

The **quantitative** results (OD values) were analyzed for statistical outliers which were evaluated using the median absolute deviation for global assessment and the Grubbs outlier test. The average OD values obtained were consistent between lots compared within a system. Outlier values were not always discordant. No specific trend (system, operator, sample or lot) was observed for outlier results. The same pattern of sample reactivity was observed between systems.

E. Performance at Low Positive Levels

The Performance at Low Analyte (Positive) Levels study was conducted to compare the performance of the assay on the new GEMINI system with the performance on the existing QuickStep system at low analyte levels. For DonorScreen-HLA, a low analyte level refers to a sample that is a low positive for HLA antibody reactivity. The HLA Class I assay and the HLA Class II assay were evaluated and tested separately.

The study included 5 samples each for the HLA Class I assay and the HLA Class II assay which were categorized as low positive for HLA antibody reactivity. The study also included one negative sample for HLA Class I and one negative sample for HLA Class II antibody reactivity. The study was conducted over 5 days of testing, with 1 run conducted per day. For each run, 12 replicates of each sample were tested.

The performance with the new GEMINI system showed equivalent or fewer numbers of false negative and false positive results per HLA class (I and II) compared to the performance with the QuickStep system.

F. False Positivity Rate

The false positivity rate for negative samples was evaluated. Serum samples from non-transfused male donors were qualified for the study by showing negative results in a more specific Luminex-based HLA antibody detection assay. Samples with positive results on the GEMINI system were assigned false-positive status based on the exclusion of true positive samples from the study.

*HLA Class I*
One hundred and seventy six (176) serum samples were tested on DonorScreen-HLA Class I using the GEMINI system. Ten (10) samples showed a positive qualitative result. False positive results were observed for 10/176 samples, or 5.7%.

*HLA Class II*
One hundred and sixty four (164) serum samples were tested on DonorScreen-HLA Class II using the GEMINI system. Two (2) samples showed a positive qualitative result. False positive results were observed for 2/164 samples, or 1.2%.

G. Plasma vs. Serum Comparison

Forty paired serum and EDTA plasma specimens were collected from random blood donors. Specimens included in the sample panel were selected to represent both HLA antibody positive and antibody negative reactivity covering a range of OD values up to approximately 1.5 OD (on a scale of 0.0 – 3.0 OD). Selection was based on historical testing of the serum samples. Plasma was not pre-tested in order to avoid selection bias.

The HLA Class I assay and the HLA Class II assay were evaluated and tested separately. Each paired run on the QuickStep and GEMINI used the same preparation of all reagents and the same aliquot(s) of each sample.

The data demonstrates there is agreement between systems. The results of the study

showed that the use of the GEMINI system compared with the use of the QuickStep system is not impacted by the sample type serum or EDTA plasma. Results of samples tested with the GEMINI system that were concordant (or discordant) between serum and plasma sample types were also concordant (or discordant) with the QuickStep system. Discordant samples were only observed with OD values close to the cutoff. The study met acceptance criteria as stated.

HLA Class I

- 2 total discordant serum/plasma results on both the GEMINI and on the QuickStep (same 2 samples across systems)
- 40/40 serum sample results were in agreement
- 40/40 plasma sample results were in agreement.

HLA Class II

- 3 total discordant serum/plasma results on the GEMINI and on the QuickStep (same 3 samples)
- 5 total discordant serum/plasma results on the QuickStep.
- 38/40 serum sample results were in agreement
- 38/40 plasma results were in agreement.
- Between system agreement was reduced because of the 2 additional discordant samples on the QuickStep and due to one sample discordant for sample type on both systems showing inverse pos/neg and neg/pos results between systems.

### 2. Stopping Solution Change 3M NaOH to Non-hazardous EDTA

A non-hazardous EDTA based solution was implemented in the assays in July 2009. During design of an alternate ELISA product, an alternate Stopping Solution was identified which eliminated the hazards of the 3M Sodium Hydroxide Stopping Solution. Studies were performed to validate the new solution.

The validation of the new solution was performed to include the following studies: effectivity to STOP a reaction in an ELISA assay, stability studies, process consistency (lot-to-lot) and functional testing in the DonorScreen-HLA® Class I and Class II assay. Forty known samples were tested in the DonorScreen-HLA® Class I and Class II assay with three separate lots of the Non-hazardous EDTA Stopping Solution. The testing showed that the non-hazardous EDTA solution effectively STOPs ELISA reactions. Stability was determined to be 5 years from date of manufacture. All forty known samples tested in the comparative studies resulted as expected with 100% concurrence of Negative or Positive qualitative values.

### 3. Additional Studies to support Assay Performance

- Alerts and Alarms

Testing to verify alerts, alarms, and error messages which users are likely to encounter with the GEMINI instrument was conducted.

The GEMINI system alerts and alarms, as well as result report flags, which were tested were triggered under the conditions expected and are described in the GEMINI Instructions for Use. The GEMINI Instructions For Use are provided in the MISC FILES Section of the electronic submission copy. The GEMINI Instructions For Use is a large file which was preclusive to include as a .pdf version in the Proposed Labeling Section (Section 13.0). Description of this testing is provided in Section 15.0, Analytical Performance Studies.

- Diluted Conjugate and Diluted PNPP in-use stability added to IFU.

The stability of Diluted Conjugate and Diluted PNPP was identified through studies performed showing that the diluted reagents may be used for up to 8 hours at room temperature (21-26°C). This covers the period of time that the instrument may be set up for a work shift.

Description of these studies is provided in Section 15.0, Analytical Performance Studies.

## VIII. Conclusion:

Based on the analytical and clinical performance studies, the data demonstrate that the assay migration to the STRATEC BIOMEDICAL AG® GEMINI instrument, the reagent change for the Stopping Solution (non-hazardous EDTA solution), assay file modification, and addition of in-process stability for two kit reagents does not present new issues of safety and effectiveness.