510(k) Summary

I. Submitter:

Owner's Name:	Genetic Testing Institute, Inc. (GTI)
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II. Name of Device:

Device Name:	™DonorScreen HLA Class I and Class II Antibody Detection Assay
Proprietary Name:	™Donor Screen HLA Class I and Class II
Classification Name:	Unclassified
Product Code:	None

III. Name of predicate devices for claiming equivalence

GTI QuikScreen®	(BK 950005)
GTI B-Screen®	(BK 990043)

IV. Description of Device:

™DonorScreen HLA Class I and Class I assay is an Enzyme Linked Immunosorbent Assay (ELISA) designed to detect anti-HLA class I and class I antibodies in blood donors. The DonorScreen assay is intended to be used on the Biotest QuickStep® instrument, which is an automated EIA microtiter plate analyzer. The QuickStep® is designed to perform the complete sample processing of qualitative and semi-

quantitative assays including the following steps: sample dilution, sample and reagent dispensing, incubations, wash processes, and plate transport. In addition the QuickStep® instrument performs photometric measurement and evaluation of the results. All of the reagents required to perform the assay on the QuickStep® instrument are provided in the DonorScreen HLA kit.

Human Leukocyte Antigens (HLAs) are highly polymorphic glycoproteins. Consequently, as a result of pregnancy, transfusions, or allograft transplantation, approximately 33% of individuals will develop antibodies to HLA. 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 been implicated in Transfusion-Related Acute Lung Injury (TRALI) and TRALI-like transfusion reactions in the recipients of blood products from the donors.

In November 2006, the American Association of Blood Banks (AABB) made recommendations that upon implementation were intended to reduce the incidence of TRALI (AABB Bulletin #06-07). One of the recommendations included implementation of interventions to minimize the preparation 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 for the presence of anti-leukoctye antibodies including anti-HLA class I and class II antibodies as well as anti-neutrophil antibodies. The AABB recommended that blood collection and transfusion facilities begin implementation of these TRALI risk reduction measures for all high plasma-volume components as soon as possible according to the schedule published in Bulletin #06-07 (November 2007 for plasma components and whole blood, and full implementation of the measures relating to platelet components as soon as possible but no later than November 2008).

Although FDA has not issued any recommendations or requirements to screen blood donors for HLA antibodies, in response to our customers' requests for a product that could be used to partially address the AABB recommendations, GTI has designed DonorScreen HLA Class I and Class II. This product is a modification of two of GTI's products that have been in use in the HLA labs for many years (12 years for QuikScreen® and 7 years for B-Screen®). Both QuikScreen® and B-Screen® have previously been cleared by FDA and serve as the predicate devices for the DonorScreen HLA assay. Both DonorScreen HLA and the predicate devices (QuikScreen® and B-Screen®) are intended to detect antibodies to HLA class I (QuikScreen®) and class II (B-Screen®).

The modifications included in the design of the DonorScreen HLA kit include automation on the QuickStep® instrument, re-packaging to minimize the reagent preparation by the user, bar-coding of the kit components and minor material changes.

The packaging modification includes combining the existing reagents from the QuikScreen® and B-Screen® kits into a single kit. In addition, the volume of the liquid reagents provided in the kit has been changed to minimize reagent preparation by the user and to provide volume required to test 176 samples in a single run on the QuickStep® instrument. Most of the kit components have bar coded labels.

There are only slight differences in the materials used in the DonorScreen HLA kit and the predicate devices. DonorScreen HLA and the predicate devices both use a pool of affinity purified HLA as the source of the antigen in the microwell. The process for making the antigen and microwell plates is identical between the products. The antigen for the DonorScreen HLA Class I microwells is derived from the platelets which represent the typical donor population. These platelets are primarily from Caucasian donors, but are not ethnically defined. The antigen for the QuikScreen is derived from a pool of platelet donors that evenly represents various ethnically defined groups including White, Black, and Hispanic donors. The antigen for the DonorScreen HLA Class II microwells is identical to that used in B-Screen® and is derived from a defined panel of EBV transformed B-lymphocyte cell lines. The lymphocyte cell lines are carefully chosen to provide a wide range of HLA class II antigens.

The remaining kit components used in the DonorScreen HLA assay are identical to the components of the QuikScreen® kit.

V. Principles of Operation

The assay steps are performed by the QuickStep® instrument and are designed to replicate the steps of the manual assays (QuikScreen® and B-Screen®). The assay parameters are pre-set by GTI and are password protected.

The principle of the assay is the same as that of the manual kits and includes the following steps: Samples are placed directly onto the instrument and may be processed directly from the primary draw tube or a number of other tube types. The sample is diluted with Specimen Diluent (1:2) and is then added to the microwells. After a 30 minute incubation at 37°C, the wells are washed with the wash buffer and any unbound antibody is removed. Diluted goat anti-human IgG – alkaline phosphatase enzyme conjugate is added to the microwell and incubated for 30 minutes at 37°C. After the

incubation step the wells are washed to remove any unbound conjugate. An alkaline phosphatase substrate, PNPP, is added to the wells. After a 10 minute incubation (Class I) or a 15 minute incubation (Class II) at room temperature the reaction is stopped by addition of the stopping solution. The optical density of the color that develops is measured at 405 nm with a 492 nm reference wavelength.

VI. Intended Use

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

VII. Support of substantial equivalence based on comparison of features, characteristics and components to the predicate device:

The following table provides a comparison between the GTI DonorScreen HLA Class I and Class II kit and the predicate devices the QuikScreen® and B-Screen® kits. The GTI QuikScreen® and B-Screen® kits are previously FDA cleared legally marketed devices (BK 950005 and BK 990043 respectively).

	DonorScreen HLA Class I and Class II	GTI QuikScreen®	GTI B-Screen®
Intended Use	DonorScreen HLA Class I and Class II is a qualitative Enzyme Linked Immunosorbent Assay (ELISA) for use on the Biotest QuickStep® instrument. DonorScreen HLA Class I and Class II ELISA is designed to detect anti-HLA class I and class II antibodies in human serum or plasma of blood donors.	QuikScreen® is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to HLA Class I antigens.	B-Screen® is a qualitative solid phase enzyme linked immunosorbent assay (ELISA) designed to detect IgG antibodies to HLA Class II antigens.
	DonorScreen HLA Class I and Class II	GTI QuikScreen®	GTI B-Screen®

Indications for Use	DonorScreen HLA Class I and Class II is designed as a solid phase enzyme linked immunosorbent assay (ELISA) for use on the Biotest QuickStep® instrument. This product is intended to be used as an *in vitro* diagnostic kit in blood bank laboratories, to screen blood donors for the presence of HLA Class I and Class II antibodies.	None cited in the original 510(k) document for QuikScreen®	(Cited as Clinical Utility in the original 510(k) document for B-Screen®): B-Screen Solid Phase ELISA Antibody Screening Kit is an adjunct, qualitative test used by physicians in evaluating patients who may have been sensitized with HLA antigens due to transfusion, pregnancy or organ transplantation for the presence of anti-HLA class II specific antibodies. The results of this test may be used with other clinical signs and test results to differentiate among immune diseases of other etiology and to aid in the determination of graft vs host disease.
Technology	ELISA with a colorimetric measurement system	ELISA with a colorimetric measurement system	ELISA with a colorimetric measurement system
Reportable Results	Qualitative assay; results are reported as positive or negative	Qualitative assay; results are reported as positive or negative	Qualitative assay; results are reported as positive or negative
Sample Type	Serum or EDTA plasma	Serum or plasma (EDTA, ACD, citrate, heparin)	Serum

KIT COMPONENTS	DonorScreen HLA Class I and Class II	GTI QuikScreen® (Class I)	GTI B-Screen® (Class II)
Microwell strips	For Class I: Immobilized affinity purified pooled HLA Class I proteins from platelet donors. The antigens represent primarily Caucasian donors. For Class II: Immobilized affinity purified antigen derived from a defined panel of EBV transformed B-lymphocyte cell lines	Immobilized affinity purified pooled HLA class I proteins from platelet donors. The antigens represent an even distribution of ethnic groups including White, Black, and Hispanic donors	Immobilized affinity purified HLA class II proteins derived from a defined panel of EBV transformed B-lymphocyte cell lines
Concentrated Wash Solution	10X Tris Buffer containing NaCl, Tween 20, and 1% NaN_3, pH 7.2	10X Tris Buffer containing NaCl, Tween 20, and 1% NaN_3, pH 7.2	10X Tris Buffer containing NaCl, Tween 20, and 1% NaN_3, pH 7.2
Specimen Diluent	Phosphate Buffered Saline, BSA, and 0.05% NaN_3	Phosphate Buffered Saline, BSA, and 0.05% NaN_3	Phosphate Buffered Saline, BSA, mouse serum, and 0.05% NaN_3
Conjugate Diluent	Phosphate Buffered Saline, BSA, and 0.05% NaN_3	Phosphate Buffered Saline, BSA, and 0.05% NaN_3	Phosphate Buffered Saline, BSA, mouse serum and 0.05% NaN_3
Substrate Buffer	Diethanolamine and magnesium chloride, 0.02% NaN_3	Diethanolamine and magnesium chloride, 0.02% NaN_3	Diethanolamine and magnesium chloride, 0.02% NaN_3
Conjugate	Goat anti-human IgG conjugated to alkaline phosphatase enzyme in a stabilizer + 0.1% NaN_3	Goat anti-human IgG conjugated to alkaline phosphatase enzyme in a stabilizer + 0.1% NaN_3	Goat anti-human IgG conjugated to alkaline phosphatase enzyme in a stabilizer + 0.1% NaN_3
Substrate	PNPP (crystalline powder)	PNPP (crystalline powder)	PNPP (crystalline powder)
Stopping Solution	3 M NaOH	3 M NaOH	3 M NaOH
Positive Serum Control	Human serum containing antibodies to HLA Class I or Class II and 0.1% NaN_3	Human serum containing antibodies to HLA Class I and 0.1% NaN_3	Human serum containing antibodies to HLA Class II and 0.1% NaN_3
Negative Serum Control	Normal Human serum containing 0.1% NaN_3	Normal Human serum containing 0.1% NaN_3	Normal Human serum containing 0.1% NaN_3

The similarities between DonorScreen HLA Class I and Class II and the predicate devices can be summarized as follows:

1. DonorScreen HLA Class I and Class II has a similar intended use to the predicate devices (all are designed for the detection of anti-HLA class I or class II antibodies).

2. DonorScreen HLA Class I and Class II utilizes the same technology (ELISA) and assay steps as the predicate devices.

3. DonorScreen HLA Class I and Class II utilizes the same assay components as the predicate devices.

4. DonorScreen HLA Class I and Class II and QuikScreen® use serum and plasma as sample types.

The differences between DonorScreen HLA Class I and Class II and the predicate devices can be summarized as follows:

1. The DonorScreen HLA Class I and Class II assay is intended to be used on an automated ELISA analyzer (Biotest QuickStep®), while the predicate devices are intended to be used manually.

2. The DonorScreen HLA Class I and Class II has a different indication for use than the predicate devices. DonorScreen HLA Class I and Class II is intended to be used to screen blood donors for the presence of HLA Class I or Class II antibodies. The predicate devices are both intended to be used for screening of transplant recipients for the presence of HLA antibodies.

3. DonorScreen HLA Class I and Class II utilizes a slightly different class I antigen pool. The platelets used for the antigen purification for DonorScreen HLA Class I antigen are derived from at least 100 random platelets from whole blood donations collected from licensed blood banks from throughout the United States. These platelets are not ethnically defined. The platelets used for the antigen purification for the QuikScreen® product are derived from an evenly distributed and ethnically defined population of donors.

4. The conjugate and specimen diluents utilized in the DonorScreen HLA Class I and Class II are identical in composition to those components used in the QuikScreen® assay. However, both of these vary slightly from the composition of these components utilized in the B-Screen® assay, the difference being the presence of 5% mouse serum in the specimen diluent used in the B-Screen® assay.

VIII. Support of Substantial Equivalence with Performance Data:

The details of each of the following studies are covered in Section 8: Performance Summary and in the additional information provided. Only a brief summary of these studies is provided in this section.

Assay Precision

Description of Study:
Three serum samples of varying antibody reactivity (low, medium, and high) were obtained. The samples were stored at 4°C throughout the time of the testing. A negative sample known to be free of HLA antibodies was also obtained and stored in the same manner. Each sample was tested in duplicate in 10 separate assays.

Results and Analysis:

To obtain the imprecision of the O.D. values, the data were analyzed by ANOVA according to CLSI Document EP5-A2, Evaluation of Precision Performance of Quantitative Measurement Methods; Approved Guideline. In addition, the reportable result (positive or negative) was analyzed for within-run and between-run agreement according to CLSI Document EP12-A Vol. 22, No 14, User Protocol for Evaluation of Qualitative Test Performance; Approved Guideline.

The calculations of imprecision of the O.D. values showed that the assay demonstrated ≤ 15% cv (total imprecision) for each of the positive samples that were tested. The negative samples (which had a very low O.D. value) showed acceptable standard deviations. In the final analysis, the correct reportable result was obtained for each result of each assay for each sample tested. There was 100% agreement between all reportable results (within-run and between-run) for each sample tested.

Conclusions:

The DonorScreen assay showed acceptable assay imprecision of the O.D. values as well as the reportable results.

Comparison of Methods Studies

Three separate studies were conducted in which the DonorScreen assay was compared to both the GTI QuikScreen® and B-Screen® assays. These assays are manual versions of the DonorScreen assay and serve as the predicate devices for DonorScreen HLA. The QuikScreen® assay is designed to detect HLA class I antibodies, while the B-Screen® assay is designed to detect HLA class II antibodies.

Description of Study:

Each study was conducted using CLSI EP9-A2; Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, as a basis for the study design. The following provides a description of the studies, the results, and conclusions.

Study 1 was conducted as an internal study at GTI. In this study samples were collected from 203 random female donors in plastic red top (serum) tubes. Samples were obtained from Interstate Blood Bank, Inc. (IBBI), and were collected from various donor collection sites across the United States. The samples were shipped at 2 – 8°C to GTI within 2 days of collecting. The samples were processed at GTI by centrifugation in order to obtain the serum. The samples were then aliquoted and stored at either 2 – 8°C for up to 9 days or at -80°C prior to testing.

Study 2 was conducted as an external study at LifeBlood Mid-South Regional Blood Center located in Memphis, TN. In this study samples were collected from 264 random female donors in plastic red top (serum) tubes. The tubes were centrifuged within 24 hours of collection to obtain the serum. The centrifuged samples were stored up to 5 days at 2 – 8°C in the primary collection tube prior to testing.

Study 3 was conducted at Florida Blood Services located in St. Petersburg, FL. In this study samples were collected from 264 random female donors and 178 random male donors. Samples were collected in plastic red top (serum) tubes. The tubes were centrifuged within 48 hours of collection to obtain the serum. The centrifuged samples were then stored up to 5 days at 2 – 8°C in the primary collection tube prior to testing.

In each study, the samples were tested in the DonorScreen HLA Class I and Class II assay as well as in the QuikScreen® and B-Screen® assays in duplicate, according to the direction insert for each of the products.

Results and Analysis:

For each sample tested, the reported result (positive or negative) from the Class I portion of the DonorScreen assay was compared to the result from the QuickScreen® assay and the reported result from the Class II portion of the DonorScreen assay was compared to the result of the B-Screen® assay.

The data from the internal method comparison study performed at GTI and the method comparison studies performed at LifeBlood Blood Center and Florida Blood Services were combined (n = 909). The combined data were then analyzed using 2x2 tables. The 2x2 table analysis of the combined data for the comparison of the DonorScreen HLA class I assay and QuikScreen® is shown in Figure 1 (below). The co-positivity was calculated to be 98.2% (95% confidence interval = 93.6 – 99.5%) and the co-negativity 100.0% (95% confidence interval = 99.5 – 100.0%). The overall agreement was 99.8%.

The 2x2 table analysis of the combined data for the comparison of the DonorScreen HLA class I and BScreen® is shown in Figure 2 (below). The co-positivity was calculated to be 97.8% (95% confidence interval = 92.3 – 99.4%) and the co-negativity 99.6% (95% confidence interval = 98.9 – 99.9%). The overall agreement was 99.4%.

Figure 1

DonorScreen HLA Class I	QuikScreen (HLA Class I) Positive	Negative	Total
Positive	108	0	108
Negative	2	799	801
Total	110	799	909

Figure 2

DonorScreen HLA Class II	BScreen (HLA Class II) Positive	Negative	Total
Positive	88	3	91
Negative	2	816	818
Total	90	819	909

Conclusions:

The data from the combined method comparison studies demonstrated that the DonorScreen HLA assay showed good sensitivity, specificity, and overall agreement with the predicate devices QuikScreen® and BScreen®.

DonorScreen HLA Class I and Class II Study using Non-Transfused Male Donors

The purpose of this study was to determine the number of false positives that might be obtained in the DonorScreen HLA assay in a sample set that should not contain HLA antibodies. The samples used in this study were obtained from male donors who stated that they had not had a transfusion in the past 12 months.

Description of Study:

In this study, 176 samples from non-transfused male donors (male donors who stated that they did not have a blood transfusion within the last year), were obtained from InterState Blood Bank, Inc. (IBBI). The samples were collected as serum in a plastic red top tube. After clot formation the samples were centrifuged to separate cells and serum per the tube manufacturer's instructions. The original collection tube was loaded directly on the

QuickStep® instrument and tested for class I and class II HLA antibodies in DonorScreen HLA Class I and Class II. This study was conducted as an internal study at GTI.

Results and Analysis:

Of the 176 samples tested, there were 9 samples that showed a positive result for class I and 4 that showed a positive result for class II. Since the sample population was non-transfused male donors, this number of positive results was un-expected.

To further study these samples to determine if they did indeed contain HLA antibodies, the samples were tested in an HLA antibody identification assay. The presence of a defined antibody specificity(ies) would indicate that these samples did indeed contain HLA antibodies. The products used for this study were HLA antibody detection and identification assays designed for the Luminex platform. The Luminex technology is reported to be a more sensitive technology than ELISA.

Results from DonorScreen HLA Class I

Of the 9 samples that were found to be positive in DonorScreen Class I, 8 of these also had a positive result in the Luminex assay and showed defined antibody specificities. Only one sample gave a negative result in the Luminex assay. This sample was a low positive in DonorScreen Class I assay with an OD value just slightly above the cutoff.

Based on the Luminex results, this sample set contained 168 true negatives (176 minus 8). Of these 168 true negatives, in the DonorScreen HLA Class I assay one sample was detected as positive, resulting in a false positive rate of 0.6% [(1/168)*100].

Results from DonorScreen HLA Class II

Of the 4 samples found to be positive in DonorScreen Class II, all had a positive result in the Luminex assay and showed defined antibody specificities. Based on the Luminex results, this sample set contained 172 true negatives for Class II (176 minus 4). Of these 172 true negatives, in the DonorScreen HLA Class II assay each of these was also negative, resulting in a false positive rate of 0%.

Conclusions:

The DonorScreen HLA Class I and Class II demonstrated false positive rates of less that 1%, which was found to be acceptable.

Interfering Substances Testing

A study was conducted to determine the effect of the following endogenous compounds in the DonorScreen HLA assay: hemoglobin, triglycerides, and bilirubin.

Description of Study:

The testing was performed using CLSI EP7, Interference Testing in Clinical Chemistry; Approved Guideline, as a basis for the study design. Briefly, the test substance was spiked into a negative sample that contained no HLA antibodies, and 3 positive samples that contained varying reactivity (low, medium, and high) of HLA antibodies. An equivalent aliquot of reagent grade water or 0.1 N NaOH (used to prepare the hemoglobin and triglyceride or the bilirubin respectively) was also spiked into a separate sample and was used as the control.

The final concentration of hemoglobin or triglyceride in the test sample was 500 mg/dL and the final concentration of bilirubin in the test sample was 20 mg/dL. Intralipid® was used as the source of triglycerides.

Samples containing the hemoglobin, Intralipid® and bilirubin and the control samples were tested in replicates of 5 using the DonorScreen reagents. This testing was performed manually using all of the DonorScreen HLA reagents for ease of experimentation. This was acceptable as there are no concerns with sample or reagent carryover on the QuickStep® instrument since each pipette tip is only used once.

Results and Analysis:

The data were analyzed in the following manner: The mean of the 5 replicates was obtained for each of the samples spiked with the test material as well as the control. The mean of the test was then compared with the mean of the control and the percent difference was obtained. In each case, there was no significant difference (<10%) in the mean O.D. value obtained for each of the test samples spiked with 500 mg/dL of hemoglobin or Intralipid® or 20 mg/dL bilirubin when compared to the corresponding controls. The percent differences observed were within the precision estimates of the assay. In addition, the presence of hemoglobin, Intralipid® or bilirubin in the test sample did not affect the reported result (the correct reportable result was obtained for the test samples spiked with these test material).

Conclusions:

Hemoglobin and triglycerides at 500 mg/dL and bilirubin at 20 mg/dL do not interfere in the DonorScreen HLA assay.

Stability Summary

No additional stability studies were conducted on the DonorScreen kit. All of the components of the DonorScreen kit are identical to those of the QuikScreen® and B-Screen® kits for which stability of each component has already been established (each component has a minimum stability of 2 years). These kits have been previously cleared by FDA, (GTI QuikScreen®; BK 950005 and GTI B-Screen®; BK 990043). The expiration of the DonorScreen kit is determined by the expiration dating of the component with the shortest dating.

IX. Conclusion:

Based on comparison with the predicate device (QuikScreen® and B-Screen®) these data demonstrate that DonorScreen HLA Class I and Class II performs comparable to the predicate devices and the DonorScreen HLA Class I and Class II kit does not present new issues of safety and effectiveness.