IMMUCOR

DonorScreen-HLA® Class I and Class II assay

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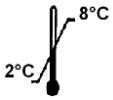

For use with the STRATEC BIOMEDICAL AG® GEMINI instrument

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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.

SUMMARY AND EXPLANATION OF THE TEST

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[1]. The formation of these antibodies in a transfusion or transplant recipient can result in the immune destruction of transfused platelets or the transplanted organ[2]. 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[3-14]. 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).

DONORSCREEN-HLA has been specifically designed to be used on the GEMINI automated ELISA instrument for the detection of class I or class II HLA antibodies in blood donors.

PRINCIPLES OF THE PROCEDURE

All assay steps described below including sample dilution, sample and reagent addition, plate washing, photometric analysis and data evaluation are carried out by the GEMINI automated ELISA instrument. Minimal reagent preparation by the user is required (refer to PROCEDURE section).

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.

REAGENTS

Maximum number of tests per kit:

- **CLASS I: 176 tests per kit**
- **CLASS II: 176 tests per kit**

Minimum number of tests per plate:

- **Each assay plate tested must contain at least 24 samples. Tap water (not reagent grade) may be used in place of samples to achieve the minimum number of samples per plate.**

There are sufficient reagents provided in the kit for 2 runs on the GEMINI instrument.
All reagents should be stored as directed by the label.

	REF	
MSI	404543	Microwell Strips: Flat-bottom microwell strips to which affinity purified HLA class I glycoproteins have been immobilized. The microwell strips are enclosed in a foil pouch. Wells are color-coded black. Ready for use. Single use.
MSII	404544	Microwell Strips: Flat-bottom microwell strips to which affinity purified HLA class II glycoproteins have been immobilized. The microwell strips are enclosed in a foil pouch. Wells are color-coded pink. Ready for use. Single use.
CWD	404563	Concentrated Wash (10X): Tris (hydroxymethyl) aminomethane buffered solution containing sodium chloride, Tween 20, and 1% sodium azide. Dilute with deionized or distilled water before use. Store Working Wash solution up to 48 hours at room temperature or up to seven days at 2 to 8°C.
SD	404581	Specimen Diluent: Phosphate buffered saline solution containing bovine albumin, and 0.1% sodium azide. White cap. Ready for use.

SB	404586	Substrate Buffer: This solution contains diethanolamine, magnesium chloride, and 0.02% sodium azide. Ready for use. Single use.
SSD	404584	Stopping Solution: Ready for use.
AG	404554	Anti-Human IgG Conjugate: Alkaline phosphatase conjugated goat affinity purified antibody to human immunoglobulin G (IgG), and 0.1% sodium azide. Blue cap. Dilute in Conjugate Diluent before use.
CDD	404564	Conjugate Diluent: Phosphate buffered saline solution containing bovine albumin, and 0.1% sodium azide. Blue cap. Ready for use. Single use.
PN	403594	PNPP Substrate: (p-nitrophenyl phosphate) Crystalline powder. Reconstitute with deionized or distilled water and dilute in Substrate Buffer before use. Protect from light. Single use.
PC I	404602	Positive Serum Control-Class I: Human serum containing 0.1% sodium azide. Black cap. Ready for use.
NC I	404591	Negative Serum Control-Class I: Human serum containing 0.1% sodium azide. Gray cap. Ready for use.
PC II	404603	Positive Serum Control-Class II: Human serum containing 0.1% sodium azide. Red cap. Ready for use.
NC II	404592	Negative Serum Control-Class II: Human serum containing 0.1% sodium azide. Pink cap. Ready for use.

PRECAUTIONS

- Do not use reagents that are turbid or contaminated.
- Care must be taken to avoid contamination of Specimen Diluent and Conjugate Diluent. Inadvertent contamination of these reagents with human serum or plasma will result in the neutralization of the conjugate and subsequently to test failure.
- Do not use reagents beyond their expiration date.
- When making dilutions, follow pipet manufacturer's instructions for appropriate dispensing and rinsing techniques.
- Microwells and reagents contained in the kit are not to be used in conjunction with any other test system.
- Discard any unused portions of diluted Conjugate, Substrate Buffer and reconstituted PNPP reagent after each run.
- The Anti-Human IgG Conjugate, Positive and Negative Controls may be used twice. When re-capping after use, be certain to place the cap onto the correct associated vial. The caps are color coded to help avoid errors when re-capping.
- The GEMINI instrument should be maintained according to the manufacturer's recommendations to ensure proper functioning.
- Place the Control rack in the loading bay lane farthest at the right. Load the rack using the positions at the rear of the instrument first. This practice reduces potential for instrument damage during pipetting from tubes containing low volume (low liquid level).
- Prior to loading, inspect samples and reagents for surface bubbles. Clear bubbles as needed to reduce potential for inaccurate pipetting.
- Per the instrument manufacturer, it is not possible to detect liquids with low ionic strengths. Recommend the use of tap water, not reagent grade water, when substituting water instead of a sample.

CAUTION

- All human serum used in the Positive and Negative controls was found negative when tested in accordance with current FDA required tests. No known test method can offer assurance that products derived from human blood will not transmit infectious agents. Therefore all blood products should be treated as potentially infectious.
- Some of the reagents in this kit or in accessory kits contain sodium azide as a preservative. WARNING: Sodium azide reacts with lead and copper plumbing forming highly explosive metal azides. When discarded in a sink, the sink should be flushed with a large volume of water to prevent azide buildup. Sodium azide is a poison and is toxic if ingested.
- Discard all components when completed according to local regulations.

INSTRUMENTATION

The STRATEC BIOMEDICAL AG® GEMINI instrument is to be used for performing the DonorScreen-HLA Class I and Class II assay. The GEMINI instrument is a fully automated microplate analyzer and includes such functions as sample dilution, sample and reagent addition, plate washing, photometric analysis and data evaluation.

Installation of the GEMINI instrument is performed by Immucor. The assay files required to use the DonorScreen-HLA Class I and Class II assay are installed by Immucor and are password protected files which cannot be modified by the user.

The instrument operating instructions, safety instructions, and a list of error codes are provided in the GEMINI instrument Instructions for Use provided with the instrument. Specific information on the instrument operating instructions regarding the set up and processing of the DonorScreen-HLA assay on the GEMINI can be found in the DonorScreen-HLA assay GEMINI Basic Operation Guide.

The user should contact Immucor for any product related concern (both GEMINI instrument and DonorScreen-HLA Class I and Class II assay product concerns). Product specific service for the DonorScreen-HLA Class I and Class II assay as well as instrument service will be provided by Immucor. Preventative maintenance of the GEMINI instrument is performed by Immucor.

SPECIMEN COLLECTION AND STORAGE

Blood should be collected in either EDTA (plasma) or without anticoagulant (serum) using aseptic technique. The primary collection tubes may be stored up to 96 hours at room temperature (21 to 26°C) or at 2 to 8°C before centrifuging to obtain the serum or plasma. To obtain the serum or plasma, the tubes should be centrifuged according to the instructions from the manufacturer of the collection tube. Once centrifuged, the sample may be tested immediately, directly from the primary tube. If not tested immediately, the samples may be stored up to 5 days at 2 to 8°C in the primary collection tube. Alternatively, the serum or plasma can be transferred to a separate tube for storage. Once transferred, samples can be stored up to 1 week at 2 to 8°C or can be frozen at -20°C or below for up to 3 years. To avoid multiple freeze/thaw cycles, it is recommended that the sample be aliquoted in small volumes and then stored frozen. Avoid frost free freezers.

Particulates or aggregates in the sample can cause false positive results. Samples containing particulates, or those that have been frozen should be clarified by centrifugation prior to testing.

PROCEDURE

Materials provided

Unless indicated some vials may contain more reagent than described on the label.

1. 2 microwell frames Class I, each containing 12-1x8 microwell strips; color coded black
2. 2 microwell frames Class II, each containing 12-1x8 microwell strips; color coded pink
3. 2 x 80 mL Concentrated Wash (10X)
4. 1 x 22 mL Specimen Diluent (white cap)
5. 2 x 25 mL Substrate Buffer (exact fill)
6. 1 x 50 mL Stopping Solution
7. 1 x 350 µL Anti-Human IgG Conjugate (blue cap)
8. 2 x 14 mL Conjugate Diluent (exact fill)
9. 2 x 50 mg PNPP Substrate
10. 1 x 250 µL Positive Serum Control-Class I (black cap)
11. 1 x 350 µL Negative Serum Control-Class I (gray cap)
12. 1 x 250 µL Positive Serum Control-Class II (red cap)
13. 1 x 350 µL Negative Serum Control-Class II (pink cap)

Additional Materials Required

1. Adjustable micropipettes to deliver 100-1000 µL and disposable tips
2. Deionized or distilled water
3. Centrifuge for centrifugation of primary collection tubes
4. STRATEC BIOMEDICAL AG® GEMINI instrument
5. Graphite tips 300 µL for GEMINI
6. Graphite tips 1100 µL for GEMINI

Test Procedure

Refer to the GEMINI Instructions for Use for information regarding use of the instrument.

1. Bring all needed reagents to room temperature.

2. Start the GEMINI system. Proceed with an assay run only if all of the self-check parameters are marked “passed”.

3. Make Working Wash solution by diluting Concentrated Wash (10x). Add 1 volume of Concentrated Wash to 9 volumes of deionized water. **Mix well**. If testing the minimum 24 samples per plate, recommend preparing at least 580 mL wash buffer. If testing the maximum 88 samples per plate, recommend preparing 750 mL wash buffer.

4. Uncap the Control Sera vials (PCI, NCI, PCII, NCII) and place into the designated positions with adapters at the end of the control rack. Ensure that barcodes face right are visible through the slot in the rack adapter. Retain the Control Sera caps.

5. Insert the control rack at track 12, the loading bay lane farthest at the right.

6. Uncap and place sample tubes into the sample racks. Ensure that any barcode label is oriented with the open slot side of the sample rack (face right) and available to the barcode reader. Barcoded and non-barcoded samples may be mixed on any rack.

NOTE 1: Various size sample tubes may be inserted in the sample rack. Ensure adequate volume is present in each tube to account for 51 µL sample aspiration. Refer to the GEMINI Instructions for Use for sample tube size guidelines.

NOTE 2: A minimum of 24 samples must be used per HLA class, requiring a minimum of 4 microwell strips per plate. If the number of samples to be tested is less than 24, tap water (not reagent grade) in a sample tube may be used instead.

7. Insert the first sample rack according to GEMINI Instructions for Use.

8. As the sample rack is read a dialogue window will open. Ensure that each sample barcode has been read. Information for non-barcoded samples can be entered at this time. For each sample select and apply the DS-Class I and DS-Class II assays (DSG-CI-ver1.0.asy and DSG-CII-ver1.0.asy, respectively).

9. After all samples and controls have been placed on the GEMINI, create a worklist.

10. Enter kit and lot information for each reagent listed. Complete the kit and reagent lot information for each plate in the appropriate fields.

11. Prepare assay reagents. Handle gently to minimize the presence of bubbles.

    a. Prepare 1 bottle of Diluted Conjugate. Make the Diluted Conjugate as follows. To 1 bottle of Conjugate Diluent add 140 µL of Anti-Human IgG Conjugate. Re-cap the bottle and mix gently by inverting several times. Once prepared, the diluted conjugate is stable for up to 8 hours at room temperature (21 to 26°C) prior to using it in the DonorScreen-HLA Class I and Class II assay.

    NOTE: The conjugate is viscous and should be thoroughly mixed with the Conjugate Diluent to assure proper performance of the assay. Un-cap and place the bottle into a small diameter position in the silver reagent rack.

    b. Re-hydrate one vial of PNPP Substrate by adding 0.5 mL deionized or distilled water to the vial. Replace the stopper and mix well. Protect from light.

    c. Prepare 1 bottle of Diluted Substrate. Make the Diluted Substrate as follows. To 1 bottle of Substrate Buffer add 250 µL of PNPP Substrate. Re-cap the bottle and mix well by inverting several times. Un-cap and place the bottle into a small diameter position in the silver reagent rack. Once prepared, the diluted PNPP substrate is stable for up to 8 hours at room temperature (21 to 26°C) prior to using it in the DonorScreen-HLA Class I and Class II assay.

    d. Un-cap and place one bottle of Stopping Solution into a small diameter position in the reagent rack. Retain the cap to seal the bottle for further use if needed.

    e. Un-cap and place one bottle of Specimen Diluent into a small diameter position in the reagent rack. Retain the cap to seal the bottle for further use if needed.

12. Start the run in the Worklist window.

13. Ensure that all the barcodes on the reagent bottles are facing right, toward the barcode reader.

14. Insert the reagent rack into the GEMINI. Ensure that there are no unallocated resources. Add Working Wash to the Wash Container (blue line) and load onto the GEMINI. Ensure sufficient Clean Fluid (deionized water) is loaded onto the GEMINI.

15. Confirm all reagents have been loaded. Check the system status Load window and load any additional pipette tips as needed. **DO NOT fill a tip rack location with the incorrect tip size. This may cause the pipettor to become contaminated or the pipettor to crash resulting in serious damage.** After ensuring that there are no unallocated resources confirm by clicking OK. A volume check of all reagents is performed automatically. If the volume of one reagent is too low, a System Error dialogue box appears. To refill a reagent or to replace it with a new bottle, the reagent rack has to be moved out, refilled or replaced and the rack re-inserted. Once re-inserted click on the “Refill Bottle” button to continue the assay.

16. Add the microwell plates as prompted. Remove the microwell plate from the foil pouch and place it into the plate carrier. If less than a full plate is required, the unused strips may be removed and returned to the resealable pouch. A unique plate ID of 20 characters or less should be entered for each plate (proposed format mmddyy-plateX).

17. Check the field immediately to the right of the Plate ID field to verify the assay name. Ensure that the correct plates are loaded for the indicated assay. DonorScreen-HLA Class I assay microwell plates are color-coded black. DonorScreen-HLA Class II assay microwell plates are color-coded pink. After confirming that the correct plate has been loaded for the assay, close the cover.

18. Once all the plates have been added and the cover has been closed, the system will start automatically.

19. Sample racks may be removed after the samples have been pipetted.

20. After processing is complete follow the instructions for unloading, disposal, and shutdown per the GEMINI Instructions for Use.

21. Unused Stopping Solution and Specimen Diluent may be re-capped and used for one additional run. Discard any remaining diluted conjugate and substrate.

22. The remainder of the control materials (Positive and Negative Serum Controls) may be re-capped and used for one additional run. Be certain to place the correct cap onto each control. The caps are color coded to help avoid errors when re-capping.

QUALITY CONTROL

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 listed below 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.

DonorScreen-HLA Class I assay Control Requirements
Mean of PC ≥ 1.500
Mean of NC $\geq$ 0.040 and $\leq$ 0.150

DonorScreen-HLA Class II assay Control Requirements
Mean of PC ≥ 1.500
Mean of NC $\geq$ 0.100 and $\leq$ 0.250

INTERPRETATION OF RESULTS

The cutoff for the DonorScreen-HLA assay is automatically calculated by the GEMINI instrument and is equal to 2x the mean of the O.D. values obtained from the Negative Control. Separate cutoff values are determined for the class I and the class II assay. A qualitative result (positive or negative) is automatically calculated and printed on the test report along with the O.D. value for each sample that is tested. The qualitative result is determined relative to the cutoff for the assay. A sample in which the O.D. value is less than the cutoff for the assay is reported as negative in the DonorScreen-HLA assay. This indicates that the sample does not contain HLA antibodies that are detectable by the DonorScreen-HLA assay. A sample in which the O.D. value is greater than, or equal to the cutoff for the assay is reported as positive in the DonorScreen- HLA assay.

LIMITATIONS

- Erroneous results can occur from bacterial contamination of test materials.
- The presence of immune complexes or other immunoglobulin aggregates in the sample may cause an increased non-specific binding and produce false-positives in this assay.
- Some low titer, low avidity antibodies may not be detected using this assay.
- This product does not detect IgM or IgA antibodies.
- This product is not intended to diagnose TRALI. Use of this test in TRALI or TRALI like reactions, which are clinical syndromes, has not been evaluated.

SPECIFIC PERFORMANCE CHARACTERISTICS

Precision

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. Assays 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 EP05-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 precision study was conducted side by side on the QuickStep and GEMINI. The imprecision of the GEMINI OD and the imprecision of the QuickStep OD values were compared as the ratio of standard deviations (GEMINI SD/QuickStep SD) for the repeatability and the total within-lab precision. Overall, the ratios of standard deviations were less than a ratio of 1 and were statistically significant. Since the ratios were less than 1 this indicated that the performance with the GEMINI system demonstrated lower standard deviations than the performance with the QuickStep system; therefore the ratios were not clinically significant. The imprecision of the GEMINI OD values met the criteria that the ratio of standard deviations in the precision study of the QuickStep and the GEMINI systems should be not statistically significant or, if statistically significant, should not be clinically significant.

Method Comparison of DonorScreen-HLA on the GEMINI system Compared to the QuickStep system

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 existing QuickStep systems. A total of 2 QuickStep and 3 GEMINI instruments were used in the study. Results were analyzed to demonstrate that the performance on the GEMINI system was comparable to the QuickStep.

The study was conducted using the Guidance for Industry and FDA Staff document entitled *Assay Migration Studies for In Vitro Diagnostic Devices* (issued April 25, 2013) as a basis for the study design. 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 same panel was tested at all 3 sites. Site 1 was an internal site which performed testing on 2 QuickStep systems and 1 GEMINI system. Site 2 was an external site which performed testing on 1 GEMINI system. Site 3 was an external site which performed testing on 1 GEMINI system.

The results of the study were analyzed for concordance using 2x2 tables. Results from the 3 GEMINI systems used at different sites were separately compared to the 2 QuickStep systems used, for a total of 6 comparisons. The following tables show the composite results from those studies by HLA class.

The acceptance criteria for the Method Comparison study were greater than or equal to 90% at the lower one-sided 95% confidence interval for the positive and negative percent agreements. The lower than expected 95% Lower CI ($\geq$ 90%) for HLA Class I negative percent agreement (NPA) was attributed to performance of the assay on one of the two QuickStep instruments used in the studies. For HLA Class I there were 48 discordant results out of 1386 test results. For HLA class II there were 25 discordant results out of 1200 test results. Discordant results occurred with samples close to the cutoff.

HLA Class I – 1,386 results from 231 samples

1386 Results		All QuickStep Instruments		% Agreement	96.5%
		Positive	Negative	Concordance (95% Lower CI)	93.9%
All Gemini Instruments	Positive	677	41	PPA (Point Estimate)	99.0%
				PPA (95% Lower CI)	96.7%
	Negative	7	661	NPA (Point Estimate)	94.2%
				NPA (95% Lower CI)	89.6%

HLA Class II – 1,200 results from 200 samples

1200 Results		All QuickStep Instruments		% Agreement	97.9%
		Positive	Negative	Concordance (95% Lower CI)	95.5%
All Gemini Instruments	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%

Method Comparison of DonorScreen-HLA on the QuickStep system to Manual ELISA QuikScreen and B-Screen

Three separate studies were conducted in which the DonorScreen-HLA Class I and Class II assay was compared to both the QuikScreen and B-Screen assays. QuikScreen is a manual ELISA which detects antibodies to HLA class I and B-Screen is a manual ELISA that detects antibodies to HLA class II.

The studies were conducted using CLSI EP9-A2; Method Comparison and Bias Estimation Using Patient Samples; Approved Guideline, as a basis for the study design. Study 1 was conducted as an internal study. In this study serum samples were collected from 203 random female donors. Study 2 was conducted as an external study. In this study serum samples were collected from 264 random female donors. Study 3 was conducted as an external study. In this study serum samples were collected from 264 random female donors and 178 random male donors. For each study the samples were tested in the DonorScreen- HLA Class I and Class II assay and the QuikScreen and B-Screen assays. The results of each study were analyzed for concordance using 2x2 tables. The DonorScreen-HLA Class I assay was compared to the QuikScreen and the DonorScreen-HLA Class II assay was compared to the B-Screen assay. The following tables show the combined results from these studies (n = 909).

The acceptance criteria for the Method Comparison study for DonorScreen-HLA Class I and Class II versus QuikScreen and B-Screen assays were $\geq$ 96% for Agreement, Co-positivity and Co-negativity. For HLA class I there were 2 discordant results out of 909 test results. For HLA class II there were 5 discordant results out of 909 test results. Discordant results occurred with samples close to the cutoff.

QuikScreen assay

DonorScreen-HLA Class I assay	**Positive**	**Negative**	**Total**
Positive	108	0	108
Negative	2	799	801
Total	110	799	909

Agreement: 99.8%
Co-positivity: 98.2% (95% Confidence Interval = 93.6 – 99.5%)
Co-negativity: 100% (95% Confidence Interval = 99.5 – 100.0%)

B-Screen assay

DonorScreen-HLA Class II assay	Positive	Negative	Total
Positive	88	3	91
Negative	2	816	818
Total	90	819	909

Agreement: 99.4%
Co-positivity: 97.8% (95% Confidence Interval = 92.3 – 99.4%)
Co-negativity: 99.6% (95% Confidence Interval = 98.9 – 99.9%)

DonorScreen-HLA Class I and Class II assay Testing using Non-Transfused Male Donors/Rate of False Positive Results

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%.

Performance at Low Analyte Levels

Assay performance at low analyte levels was verified on the GEMINI system. Testing was conducted over 5 days, using 1 negative sample and 5 low positive samples per HLA class.

For HLA Class I, the performance with the Gemini system showed 0 false negative results for each low positive sample (60 positive results/60 total results obtained for each sample). One negative sample showed one false positive result (1 positive and 59 negative results out of 60 total results).

For HLA Class II, the performance with the Gemini system showed 0 false negative results for each low positive sample (60 positive results/60 total results obtained for each sample). One negative sample showed 0 false positive results (60 negative results out of 60 total results).

Reproducibility

Reproducibility studies were performed for DonorScreen-HLA Class I and HLA Class II assays at three clinical trial sites demonstrating results obtained with the GEMINI system. Twenty four samples (triplicates of eight unique samples) covering varying reactivity for HLA class I and for HLA class II were provided to each site. Two operators at each site (6 operators total) participated in the study which consisted of two runs per day for five non-consecutive days. The Overall Comparison for the Reproducibility Study is provided in the table below.

HLA Class I

Concordance by Sample Type-Class I GEMINI							
Sample Type	**Total Tests**	**Expected Positive**	**Observed Positive**	**% (95% Lower CI)**	**Expected Negative**	**Observed Negative**	**% (95% Lower CI)**
Negative	360	0	5	n/a	360	355	98.6% (96.8%)
High Negative	360	0	10	n/a	360	350	97.2% (95.0%)
Positive/Low	360	360	360	100.0% (99.2%)	0	0	n/a
Positive/Moderate	360	360	360	100.0% (99.2%)	0	0	n/a
Total	1440	720	720	100.0% (99.6%)	720	715	99.3% (98.4%)

HLA Class II

Concordance by Sample Type-Class II GEMINI							
Sample Type	**Total Tests**	**Expected Positive**	**Observed Positive**	**% (95% Lower CI)**	**Expected Negative**	**Observed Negative**	**% (95% Lower CI)**
Negative	360	0	0	n/a	360	360	100.0% (99.2%)
High Negative	360	0	3	n/a	360	357	99.2% (97.6%)
Positive/Low	360	360	356	98.9% (97.2%)	0	4	n/a
Positive/Moderate	360	360	357	99.2% (97.6%)	0	3	n/a
Total	1440	720	713	99.0% (98.0%)	720	717	99.6% (98.8%)

Interfering Substances

Interfering Substance studies were conducted using CLSI EP7-A2 Interference Testing in Clinical Chemistry; Approved Guideline.

The following substances showed no interference in the DonorScreen-HLA Class I and Class II assay at the concentration indicated:

Hemoglobin	≤ 500 mg/dL
Triglycerides	≤ 500 mg/dL
Bilirubin	≤ 20 mg/dL

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Warning	Warning
Danger	Danger
H302	Harmful if swallowed
H318	Causes serious eye damage
H412	Harmful to aquatic life with long lasting effects
EUH032	Contact with acids liberates very toxic gas
P264	Wash hands thoroughly after handling
P270	Do not eat, drink or smoke when using this product
P273	Avoid release to the environment
P280	Wear protective gloves/protective clothing /eye protection/face protection
P301 + P312	IF SWALLOWED: Call a POISON CENTER or doctor/physician if you feel unwell
P305 + P351 + P338	IF IN EYES: Rinse cautiously with water for several minutes. Remove contact lenses, if present and easy to do. Continue rinsing
P310	Immediately call a POISON CENTER or doctor/physician
P330	Rinse mouth