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LIFECODES

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PRODUCT INSERT

LIFECODES® LSA™ Class I

LIFECODES® LSA™ Class II

For In Vitro Diagnostic Use. IVD

Rx ONLY

TABLE OF CONTENTS

Definition of Symbols	1	**Procedure**	3
Summary and Explanation	2	A. Materials Provided	3
Principles of the Procedure	2	B. Materials Required, but not Provided	3
Reagents	2	**Directions for Use**	4
A. Identification	2	**Results**	5
B. Warnings and Cautions	3	**Quality Control**	5
C. Storage Instructions	3	**Limitation of the Procedure**	5
D. Purification or Treatment for Use	3	**Troubleshooting**	6
E. Instability Indications	3	**Specific Performance Characteristics**	6
Instrument Requirements	3	**References**	12
Specimen Collection and Preparation	3		

DEFINITION OF SYMBOLS

(Product Labels and Supplemental Documents)

Batch Code	LOT	Catalog Number	REF	Use By Date		Temperature range (storage)	2°C – 8°C
SAMPLE	SAMPLE	Manufacturer		MFI Threshold	MFI TH	Temperature (storage)	15-25°C
Dilute Before Use	DIL	Keep away from light		Sufficient for N tests	Σ	Consult Instructions for Use	
Name	NAME	Identification Number	ID#	Date	DATE	Technician	TECH
Bead	BEAD	Class I	CLI	Class II	CLII	Cut-off	CUT-OFF
Background	BKG	Antigen	AG	Median Fluorescence Intensity	MFI	Interpretation	INTRP
Negative Control Bead	NC	Positive Control Bead (Immunoglobulin G)	PC	Bleed date	BDT	Antigen ID	ANTIGEN ID
Lowest Ranked Antigen	LRA	MFI / Lowest Ranked Antigen	MFI/LRA	Warning		Observed Limits	OBSERVED LIMITS
Relative Antigen Density	RAD	Serological Equivalent	SERO	Prescription Use Only	Rx ONLY	*In vitro* diagnostic medical device	IVD

Key: Underline = Addition or significant change; ▲ = Deletion of text

INTENDED USE

LIFECODES® LSA™ Class I and Class II are bead-based immunoassays used to qualitatively detect HLA IgG antibodies to aid donor and recipient matching in transfusion or transplantation. Luminex Instrument and XY Platform are required to run the LIFECODES LSA Class I and Class II assays. The MATCH IT!® Antibody Software is intended as an aid in the analysis of LIFECODES LSA Class I and Class II assays.

SUMMARY AND EXPLANATION

Human leukocyte antigens (HLA) are a system of glycoproteins that have a functional role in the presentation of peptides to the immune system.[1,2] However, as a highly polymorphic system, HLA molecules can become the targets of antibody responses in people during pregnancy, transfusion of blood products, or organ transplantation. Generally, alloimmunization leads to the production of HLA antibodies in approximately 33% of exposed individuals.[3] The presence or absence of these HLA-specific antibodies has a role in determining the survival of transplant allografts.[4]

LIFECODES LSA Class I Beads are designed to detect IgG antibodies to HLA Class I glycoproteins. LSA Class I is composed of different Luminex Beads to which purified recombinant Class I HLA glycoproteins are conjugated.

LIFECODES LSA Class II Beads are designed to detect IgG antibodies to HLA Class II glycoproteins. LSA Class II is composed of different Luminex Beads to which purified recombinant Class II HLA glycoproteins are conjugated.

PRINCIPLES OF THE PROCEDURE

An aliquot of the Beads is allowed to incubate with a small volume of test serum sample. The sensitized beads are then washed to remove unbound antibody. An anti-Human IgG antibody conjugated to phycoerythrin is then added. After another incubation, the test sample is diluted and analyzed on the Luminex instrument. The signal intensity from each bead is compared to the signal intensity of the lowest ranked locus-specific bead included in the bead preparation to determine if the bead is positive or negative for bound alloantibody.

REAGENTS

A. Identification

265100IVD: LSA1 LIFECODES® LSA™ Class I consists of five (5) components in sufficient quantities for 24 tests.

1. **265103 LSA1B LSA Class I Bead Mix** (960 µL): A blend of beads each conjugated with a different single Class I HLA glycoprotein plus control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine proteins. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. **Store at ≤ –65°C in the dark.**
2. **265002 LSACJ LSA Conjugate Concentrate** (120µL): Goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20 and sodium azide. **DIL** MUST BE DILUTED 1:10 in Wash Buffer prior to use. LIGHT SENSITIVE. Keep out of direct light for extended periods of time. **Store at 2 to 8°C in the dark.**
3. **628221 LMWB LIFECODES Wash Buffer** (30 mL): A phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine serum albumin. **Store at 2 to 8°C** and equilibrate to room temperature (20-24°C) prior to use.
4. **265101 LSAPC1 LSA Class I Positive Control** (100 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens and will react with most of the LSA Class I Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**
5. **265102 LSANC1 LSA Class I Negative Control** (100 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens and will react with few if any of the LSA Class I Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**

265200IVD: LSA2 LIFECODES® LSA™ Class II consists of five (5) components in sufficient quantities for 24 tests.

1. **265203 LSA2B LSA Class II Bead Mix** (960 µL): A blend of beads each conjugated with a different single Class II HLA glycoprotein plus control beads. The storage buffer is a phosphate-based buffer containing NaCl, Tween-20, sodium azide, and bovine proteins. LIGHT SENSITIVE. Keep routine exposure to light to three hours or less. **Store at ≤ –65°C in the dark.**
2. **265010 LSACJ LSA Conjugate Concentrate** (120µL): Goat anti-Human IgG conjugated to phycoerythrin in a phosphate-based storage buffer containing NaCl, Tween-20 and sodium azide. **DIL** MUST BE DILUTED 1:10 in Wash Buffer prior to use. LIGHT SENSITIVE. Keep out of direct light for extended periods of time. **Store at 2 to 8°C in the dark.**
3. **628221 LMWB LIFECODES Wash Buffer** (30 mL): A phosphate-based buffer containing NaCl, Tween-20, sodium azide and bovine serum albumin. **Store at 2 to 8°C** and equilibrate to room temperature (20-24°C) prior to use.
4. **265201 LSAPC2 LSA Class II Positive Control** (100 µL): This serum or sera blend is obtained from individual(s) shown to be alloimmunized to HLA antigens and will react with most of the LSA Class II Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**
5. **265202 LSANC2 LSA Class II Negative Control** (100 µL): This serum or sera blend is obtained from individual(s) known to have no antibodies to HLA antigens and will react with few if any of the LSA Class II Beads. Contains 0.1% sodium azide as a preservative. **Store at 2 to 8°C.**

B. Warnings or Cautions

1. For In Vitro Diagnostic Use
2. Due to the complex nature of HLA testing, qualified personnel should review data interpretation. The detection of antibody using LSA kits must take into consideration the results of all beads, including those that may be at or near the cut-off value. Knowledge of the sample history as well as an understanding of the cross-reactive groups can be useful in the evaluation of a specific serum.
3. The results of these tests should not be used as the sole basis for making a clinical decision.
4. The use of this device as a companion diagnostic has not been established.
5. Human source material used in the production of this kit has been tested and found to be negative for antibody to HIV, HCV, and HBsAg by FDA-approved methods. However, no test method can offer complete assurance that infectious agents are absent. Therefore, **use Universal Precautions** when working with these materials.
6. Substitution of components other than those provided in this system may lead to erroneous results.
7. Reagents contain 0.1% sodium azide as a preservative, which may react with lead and copper plumbing to form explosive metal azides. Use large amounts of water when discarding materials down a sink.
8. Bacterial contamination of samples or the presence of immune complexes or other immunoglobulin aggregates can cause increased non-specific binding and erroneous results.
9. This product detects IgG antibodies that may or may not be lymphocytotoxic.
10. This product is not expected to detect antibodies of the IgA or IgM class of immunoglobulin.
11. These products are designed for use with the Luminex instrument according to the manufacturer's recommendations.
12. Dispose of all materials after use according to local regulations.
13. See Safety Data Sheets for additional information.

C. Storage Instructions

1. Refer to product labels for storage indications.
2. Beads and conjugate are LIGHT SENSITIVE. Keep routine exposure to light to three hours or less.

D. Purification or Treatment Required for Use

1. See "Specimen Collection and Preparation."
2. Conjugate Concentrate must be diluted 1:10 in Wash Buffer before use.

E. Instability Indications

1. Do not use components or controls that are turbid or beyond their expiration date.
2. Discard all unused diluted positive and negative controls and conjugate after use.

INSTRUMENT REQUIREMENTS

Luminex Instrument and XY Platform (LIFECODES Product Number 888300, 888302) or Luminex FLEXMAP 3D (LIFECODES Product Number 888303).

SPECIMEN COLLECTION AND PREPARATION

Blood should be collected without anticoagulant using aseptic technique and should be tested while still fresh to minimize the chance of obtaining false-positive or false-negative reactions due to improper storage or contamination of the specimen. Serum should be stored at 2 to 8°C for no longer than 48 hours. If serum is to be stored beyond 48 hours, it should be frozen at or below –20°C for up to 2 years. Individual laboratories should establish and validate methods for storing sera for more than 2 years. Serum should be separated from red cells when stored or shipped. Avoid repeated freezing and thawing of serum samples.

Note: Do not use microbiologically contaminated sera. Although hemoglobin and triglycerides exhibited no inhibition in an interfering substances study, hemolyzed or lipemic sera should be avoided as these samples may give inconsistent results.

Prior to assaying, all samples should be vortexed and centrifuged briefly (30 seconds at 10,000xg) to pellet any particulate matter that may be present.

PROCEDURE

A. Materials Provided (See REAGENTS on page 2 for more specific information)

- LSA Bead Mix
- Conjugate Concentrate
- Wash Buffer
- Positive Control Serum
- Negative Control Serum
- Recording Sheet
- Plate Format Sheet

B. Materials, Reagents and Equipment *Required*, but Not Provided (as listed or equivalent)

- 5 µL – 50 µL adjustable pipets with appropriate pipet tips
- 250 µL multichannel pipet with matching tips and buffer trough
- 1.5 mL microcentrifuge tubes for conjugate dilutions
- Test tubes for patient and control samples
- Timer
- Marking pen
- Millipore multiscreen filter plates (Millipore Cat# MSBVN1210, MSBVN1250, Lifecodes Cat # 888633 or 888633-50)
- Luminex Sheath Fluid (▲Lifecodes Cat # 628005) or Luminex Sheath Fluid Plus (Lifecodes Cat # 628010)
- Multiscreen vacuum manifold (Millipore Cat # MAVM 0960R, Qiagen Cat # 19504, Lifecodes Cat # 888315)
- Luminex Calibration Kits (Luminex 100/200 Calibration Kit, Luminex 100/200 Performance Verification Kit, Lifecodes Cat # 628018 and 628019 respectively) or FLEXMAP 3D Calibration Kits (Luminex FLEXMAP 3D Calibration Kit, Luminex FLEXMAP 3D Performance Verification Kit Lifecodes Cat# 888307 and 888308 respectively
- Distilled water
- Rotary Platform
- Adhesive plastic covers (Corning Cat # 6524 or 6570)

DIRECTIONS FOR USE

PRECAUTIONS:

- Care MUST be taken to avoid contamination of Wash Buffer and the anti-Human IgG reagent. Inadvertent contamination of these reagents with human serum will result in the neutralization of anti-Human IgG and subsequently result in test failure.
- Care must be taken to control vacuum strength. Strong vacuum pressure can cause beads to stick to the membrane causing bead count failure.
- Care must be taken during pipetting into the filter plate so that beads do not stick to the side of the microplate wells. Beads should be pipetted into the well being careful not to touch the membrane with the tip. Contacting the membrane with the pipet tip can lead to puncture of the membrane and subsequent failure of the assay.
- Care must be taken to ensure, during incubation steps, that the beads are not splashing and sticking to the sides of the wells. When running the assay for the first time, run a few positive and/or negative controls to determine the optimal speed for the rotary platform. A speed of approximately 200 rotations per minute with an orbit size of 19mm has been shown to be effective.
- The presence of significant levels of unbound antibody at the completion of the wash step, due to either excess serum or poor washing, may reduce the ability of the assay to detect IgG bound to sensitized beads and cause erroneous results.
- A sample of positive and negative control sera should be included with each test to help determine if technical error or reagent failures have occurred.

1. Take out the LSA Bead Mix from the freezer and store it in the dark at room temperature until thawed. Then place on ice and protect from light. **NOTE: The bead mix can be frozen and thawed a maximum of 6 times.**
2. Leaving other components at 2 to 8°C in the dark until required, bring the Wash Buffer to room temperature (20 to 24°C) prior to use. During this time, use the Plate Format Sheet to assign a position on the plate for each of the sera and controls to be analyzed. The control sera supplied in the kit are used to illustrate a broadly reactive positive alloserum and a negative serum.
3. Cover the unassigned wells of the Filter Plate with adhesive plastic cover. Pre-wet wells to be used with 100-300 µL of distilled water. After 2-5 minutes, remove water by gentle aspiration using the vacuum manifold. (See manufacturer's recommendations for proper use.)
4. Prepare the LSA Beads by briefly (30 seconds) centrifuging the vial at 600 – 800 xg to remove any beads or liquid from the cap or walls of the vial. Thoroughly vortex (~1 minute) to evenly resuspend the beads.
5. Add 40 µL of LSA Beads to each of the assigned wells. Re-vortex the LSA Bead vial every 2 minutes to keep the beads in suspension while distributing the beads, then add 20 µL of patient serum and control sera and mix.

CAUTION: It is important to keep the beads resuspended to ensure sufficient beads are aliquoted into wells and to ensure low count times. Failure to vortex beads intermittently will cause beads to settle towards the bottom of the tube. This will result in differential amount of beads being dispensed into wells which may adversely affect run-times and analysis of results.

6. Cover the plate with adhesive plastic cover then foil or box to protect from light. Incubate for 30 minutes at room temperature (20-24°C) in the dark on a rotating platform. Return unused portions of control sera to storage at 2 to 8°C for future use. Return unused portions of LSA Bead Mix to storage at ≤ –65°C in the dark for future use.
7. Dilute conjugate with Wash Buffer (5 µL conjugate to 45 µL Wash Buffer per sample). To accommodate pipetting losses, it is desirable to make up one (1) extra volume of diluted conjugate. Cover with foil and/or store in the dark at room temperature until used. Return the unused portion of Conjugate Concentrate to storage at 2 to 8°C in the dark for future use.
8. After the 30 minute incubation remove the adhesive plastic cover and add 100 µL of Wash Buffer to each well. Mix to resuspend the beads and gently aspirate the plate.

CAUTION: Use of excessive vacuum strength will cause beads to stick to the membrane and can result in sample failure. Apply the minimum vacuum pressure required to aspirate samples.

9. Add 250 µL of Wash Buffer to each well, mix to resuspend the beads, aspirate, and repeat two more times for a total of three washes.

CAUTION: Failure to wash completely may reduce the ability of the conjugate to detect IgG bound to sensitized beads and cause false negative results.

10. Add 50 µL of diluted conjugate to each well. Cover plate with foil or box to protect from light. Place on a rotating platform or gently vortex every 5-10 minutes. Incubate for 30 minutes at room temperature (20 to 24°C).
11. Using a clean pipette tip, add 130 - 150 µL of Wash Buffer to each well and mix to resuspend beads.
12. Collect data with Luminex instrument using the LSA lot-specific Luminex Template and following the manufacturer's recommendations. Delays of greater than 3 hours may increase the chance of obtaining false-positive or false-negative reactions. Return the unused portion of Wash Buffer to storage at 2 to 8°C for future use.

RESULTS

MATCH IT!® Antibody Software is intended as an aid in the analysis of LIFECODES LSA Class I and Class II

Sample Analysis is performed as follows. Enter the Raw Median Fluorescence Intensity (MFI) values for each bead into the lot-specific Recording Sheet. To determine if a bead is positive, first establish if the MFI for each antigen bound bead is above the MFI Threshold found on the lot-specific Recording Sheet provided with the kit. If an antigen bound bead is above the MFI Threshold, divide the MFI by the MFI of the Lowest Ranked Antigen (LRA) of its respective locus to generate the MFI/Lowest Ranked Antigen (MFI/LRA) ratio. The LRA for each locus is the MFI value of the lowest ranked antigen bead for that locus.

Example:

$$\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "1"}} = \text{MFI/LRA for antigen "x" from locus "1"}$$

$$\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "2"}} = \text{MFI/LRA for antigen "y" from locus "2"}$$

$$\frac{\text{Individual Bead MFI}}{\text{LRA MFI for locus "3"}} = \text{MFI/LRA for antigen "z" from locus "3"}$$

The LIFECODES LSA Class I and Class II products detect antibodies at the antigen/allele level. Refer to the lot-specific Recording Sheet provided with the kit for the list of the antigens present on each bead and the MFI/LRA cut-off for determining the positive/negative result with each antigen bound bead. An antigen bound bead is considered positive if the MFI value is above the MFI Threshold and the MFI/LRA ratio is above the MFI/LRA cut-off value.

Example of Antigen/Allele Assignments for a Sample

MFI TH 750

BEAD	ANTIGEN ID	ANTIGEN	MFI	LRA	MFI/LRA	CUT-OFF	INTRP
4	103	A*01:01	500	350	1.43	2.56	NEG
4	104	A*02:01	5000	350	14.29	3.14	POS
6	106	A*02:02	7500	350	21.43	3.25	POS
7	107	A*02:03	4000	350	11.43	2.89	POS
8	108	A*02:05	3000	350	8.57	3.65	POS

Caution:

- To obtain reliable results, there must be sufficient data gathered by the Luminex Instrument.
- Collect at least 60 events for each bead region. This can be verified by reviewing the ***DataType: Count*** section of the csv file.

QUALITY CONTROL

Quality control of LSA Class I and Class II is built into the test system by the inclusion of Positive and Negative Control Sera. These controls must be included with each test run to help determine if technical errors or reagent failures have occurred. The Positive Control Sera will react with a large number of HLA conjugated beads generating a pattern similar to that found in the lot-specific Recording Sheet. The Negative Control Sera will be negative and react with few if any of the HLA conjugated beads generating values ≤1000 MFI.

The bead sets include two control beads to monitor each sample's performance. The Positive Control Bead is coated with human IgG and should yield MFI values ≥10,000 with the control sera. If you obtain values less than 10,000 MFI with the control sera, your assay may be insufficiently washed or your conjugate may be compromised. Clinical samples will exhibit a wide range of MFI values with the Positive Control Bead. The observed range for the Positive Control Bead in clinical studies was 524 – 24,036 MFI (interquartile range 9,721 – 20,947 MFI) for class I and 8,305 – 22,686 MFI (interquartile range 18,065 – 19,828 MFI) for class II. The Negative Control Bead should show low MFI values with the control sera. Refer to the lot specific recording sheet for observed limits for the control beads with quality control sera.

The assay should be run as recommended in the package insert as well as performed with any other quality control procedures that are in accordance with local, state, federal and/or accreditation agencies requirements.

LIMITATIONS OF THE PROCEDURE

Erroneous results can occur from bacterial contamination of test materials, inadequate incubation periods, inadequate washing or decanting of beads, exposure of conjugate to stray light, or omission of test reagents or steps.

The presence of immune complexes or other immunoglobulin aggregates in the serum sample may cause increased non-specific binding and produce erroneous results in this assay.

CAUTION: HLA-specific IgM is known to interfere through direct competition with HLA-specific IgG with identical specificities[8].

The antibodies detected by LSA Kits are those reactive within the population of available antigens listed on the Recording Sheet.

LIFECODES Single Antigen HLA Class I and II glycoproteins were obtained from cell lines expressing single HLA antigens.

Some IgG with low avidity or low titer, IgA, IgM and monospecific antibodies to antigens not included in the panel will not be detected with the LIFECODES Single Antigen assays.

Serum antibody titers are sample and time point specific. If many beads are producing MFI values above 15,000, it may be necessary to dilute the sera for better detection of IgG antibodies.

TROUBLESHOOTING

PROBLEM	POSSIBLE CAUSE	SOLUTION
Low Bead Count	Bead Mix not well suspended	Pulse vortex to completely resuspend
	Instrument failures - out of calibration	See Luminex Instrument Manual
	Instrument failures - sample flow blocked	See Luminex Instrument Manual
	Photobleached beads	Use new kit
	Vacuum pressure too strong/beads stuck to membrane	Reduce vacuum strength; Millipore Multiscreen Filter Plates recommend a vacuum of 271-406 millibar (8-12 in. Hg)
Negative Control Bead (NC) Threshold Surpassed with Control Sera	Poor washing	Repeat and monitor washes
	Incorrect sample added	Repeat with correct control sample
Positive Control Bead (PC) Threshold Failure with Control Sera	Compromised conjugate e.g. photobleaching	Use new kit
	Poor washing	Repeat and monitor washes
	Incorrect sample added	Repeat with correct control sample
Anomalous pattern for Positive Control Sera	Incorrect sample added	Repeat with correct control sample
	Poor washing	Repeat and monitor washes
Positive assignment for Negative Control Sera (>2 HLA conjugated beads) or >1000 MFI:	Incorrect sample added	Repeat with correct control sample
	Poor washing	Repeat and monitor washes to ensure beads are re-suspended during washing
		Reduce vacuum strength
	Contamination of Bead Mix, Wash Buffer, Negative Control Sera or Conjugate Concentrate with positive sample	Use new kit
Clogged filter plate	Particulate matter in sample	Centrifuge sample approximately 5 minutes at 8,000 – 12,000xg

SPECIFIC PERFORMANCE CHARACTERISTICS

Interfering Substances

The following substance showed no interference in the LIFECODES LSA1 and LSA2 assay at the concentrations indicated:

Substance	Concentration
Belatacept	0.34 mg/mL
IVIG	1.25 mg/mL
Rituximab	0.479 mg/mL
Alemtuzumab	0.021 mg/mL
ATG	170µg/mL
Eculizumab	0.27 mg/mL
Bortezomib	1.3 µg/mL
Infliximab	0.006 mg/mL
Daclizumab	0.054 mg/mL
Ethylenediaminetetraacetic acid (EDTA)	10 mM
Dithiothreitol (DTT)	10 mM
Fetal Bovine Serum (FBS), heat inactivated	18.2% (v/v)
Triglycerides	15 mg/mL
Hemoglobin	10 mg/mL
IgM	3 mg/mL

Accuracy Studies

An accuracy study was performed internally using 151 clinical samples for LSA Class I and 150 clinical samples for LSA Class II.

The LIFECODES LSA Class I kit was compared to results obtained from LABScreen Single Antigen HLA Class I – Combi, Cat #LS1A04. The comparison is based on the 84 antigens in common between the two products (Table 1A). The point estimates for overall agreement, PPA and NPA per antigen are depicted in Figure 1A.

The LIFECODES LSA Class II kit was compared to results obtained from LABScreen Single Antigen HLA Class II – Group 1, Cat #LS2A01. The comparison is based on the 63 antigens in common between the two products (Table 1B). The point estimates for overall agreement, PPA and NPA per antigen are depicted in Figure 1B.

Table 1A. Accuracy Study LSA1

		LABScreen Single Antigen HLA Class I-Combi			% Agreement	93.7%
		Positive	Negative	Total	Concordance (95% Lower CI)	93.4%
LSA I	Positive	2231	266	2497	PPA (Point Estimate)	80.9%
	Negative	528	9659	10187	PPA (95% Lower CI)	79.6%
	Total	2759	9925	12684	NPA (Point Estimate)	97.3%
					NPA (95% Lower CI)	97.0%

Figure 1A. Class I Comparison at Antigen Level

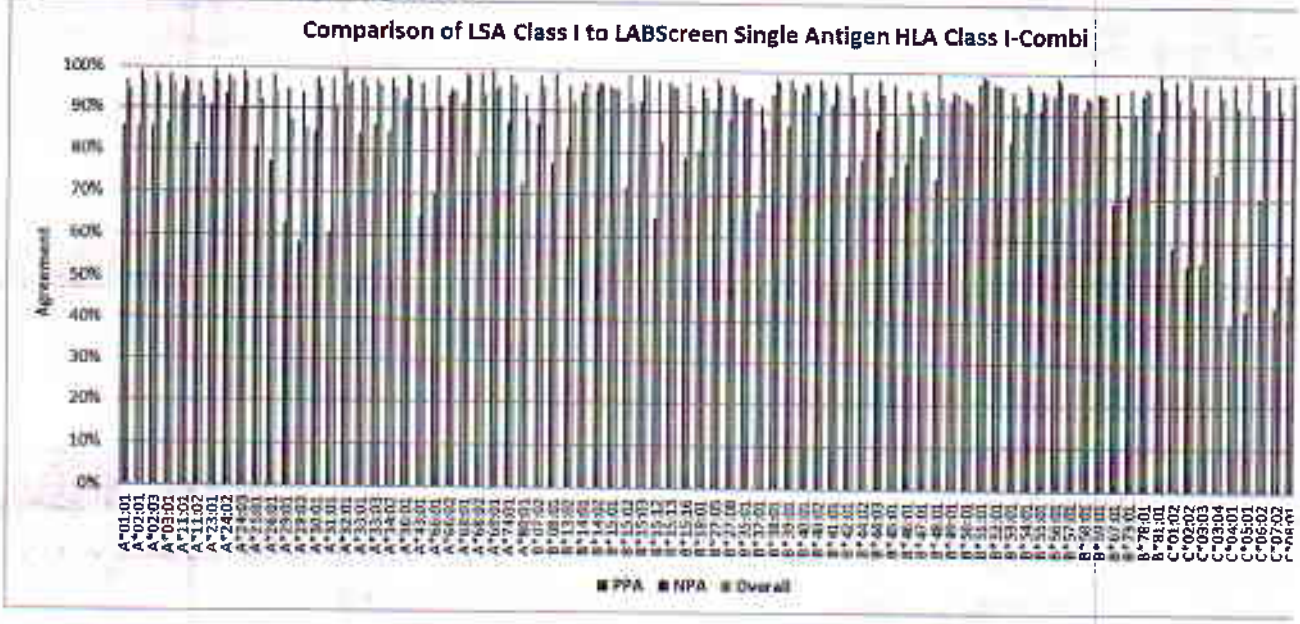
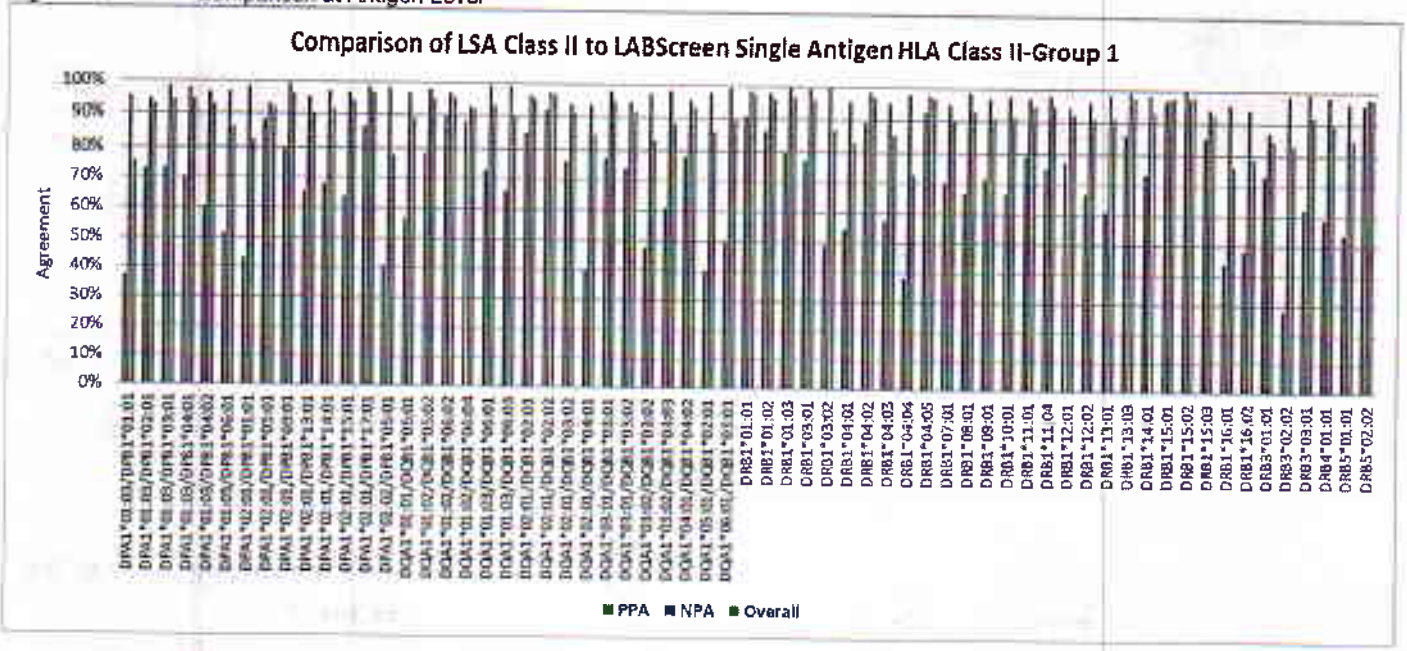


Table 1B. Accuracy Study LSA2

		LABScreen Single Antigen HLA Class II-Group 1			% Agreement	90.5 %
		Positive	Negative	Total	Concordance (95% Lower CI)	89.9 %
LSA II	Positive	1194	244	1438	PPA (Point Estimate)	64.6 %
	Negative	654	7358	8012	PPA (95% Lower CI)	63.0 %
	Total	1848	7602	9450	NPA (Point Estimate)	96.8 %
					NPA (95% Lower CI)	96.0 %

Figure 1B. Class II Comparison at Antigen Level



Reproducibility

A reproducibility study was performed at three sites to demonstrate the reproducibility of the results obtained with the LIFECODES Single Antigen (LSA) Class I and Class II kits. Each site provided two operators to participate in the study and testing consisted of two runs per day over five non-consecutive days. Seven samples from multiparous females were tested in triplicate. The samples were selected to maximize antigen coverage: there was at least one positive sample for 88 out of the 96 Class I antigens and 94 out of the 96 Class II antigens. The overall results for LSA Class I and LSA Class II are shown below in Tables 2A & 2B.

Table 2A. Overall Results for LSA Class I

		Expected Results				
		Positive	Negative	Total	% Agreement	98.09%
					Concordance (95% Lower CI)	98.03%
					PPA (Point Estimate)	97.00%
LSA I Observed Results	Positive	28272	1409	29681	PPA (95% Lower CI)	96.83%
	Negative	876	89251	90127	NPA (Point Estimate)	98.45%
	Total	29148	90660	119808	NPA (95% Lower CI)	98.38%

Table 2B. Overall Results for LSA Class II

		Expected Results				
		Positive	Negative	Total	% Agreement	99.04%
					Concordance (95% Lower CI)	98.98%
					PPA (Point Estimate)	98.65%
LSA II Observed Results	Positive	45061	725	45786	PPA (95% Lower CI)	98.56%
	Negative	615	74463	75078	NPA (Point Estimate)	99.04%
	Total	45676	75188	120864	NPA (95% Lower CI)	98.98%

A second study was performed using 94 clinical samples comparing internal results to results from an external site. The 94 samples are a sub-set of the samples used in the Accuracy Study. The LSA Class I and LSA Class II kits used in this additional study at both sites were comprised of the same reagent lots numbers. A Luminex 200 instrument with xPONENT 4.3 software was used at both sites to acquire data.

LSA Class I: Site to site comparison data demonstrated a total Percent Agreement of 96.85% (95% LCL = 96.53%) The overall PPA was 92.53% (95% LCL = 91.70%). The overall NPA was 99.11% (95% LCL = 98.88%).

LSA Class II: Site comparison data demonstrated a total Percent Agreement of 98.03% (95% LCL = 97.77%) The overall PPA was 94.30% (95% LCL = 93.36%). The overall NPA was 99.05% (95% LCL = 98.84%).

Lot-to-Lot Reproducibility was performed using 9 positive sera from multiparous females and 1 high negative serum from a non-transfused male. Each sample was run 6 times on each lot. Antigen reactivity was categorized as follows: Low Negative – MFI ≤ 500 and assigned negative; High Negative – MFI > 500 and assigned negative; Low Positive – MFI < 5000 and assigned positive; High Positive – MFI ≥ 5000 and assigned positive. The results are summarized in Tables 3A and 3B for LSA Class I and LSA Class II, respectively.

Table 3A. Lot-to-lot Reproducibility Results for LSA Class I

Reactivity	Total Tests	Lot #1	Lot #2	Lot #3	Lot #4
Low Negative	1662	100.0%	100.0%	100.0%	100.0%
High Negative	576	100.0%	100.0%	100.0%	100.0%
Low Positive	876	99.2%	100.0%	100.0%	100.0%
High Positive	1338	100.0%	100.0%	100.0%	100.0%

Table 3B. Lot-to-lot Reproducibility Results for LSA Class II

Reactivity	Total Tests	Lot #1	Lot #2	Lot #3	Lot #4	Lot #5
Low Negative	2112	100.0%	99.3%	100.0%	100.0%	100.0%
High Negative	576	100.0%	100.0%	99.8%	100.0%	98.6%
Low Positive	840	100.0%	100.0%	100.0%	99.9%	100.0%
High Positive	1182	100.0%	100.0%	100.0%	100.0%	100.0%

Clinical Studies

Method comparison studies were conducted at two (2) external sites and one (1) internal site. Immucor, Inc., as the manufacturer, was the internal site. Serum samples were tested with LIFECODES LSA kits and a comparator (LABScreen Single Antigen kits). Test results were evaluated for agreement between the reagents. LIFECODES LSA results were analyzed according to the procedure described in the Results section. All LABScreen results with scores >4 were considered positive.

The LIFECODES LSA Class I kit was compared to results obtained from LABScreen Single Antigen HLA Class I – Combi, Cat #LS1A04, using data collected on a total of 507 samples. The comparison is based on the 84 antigens in common between the two products (Table 4A). The point estimates for overall agreement, PPA and NPA per antigen are depicted in Figure 2A.
The LIFECODES LSA Class II kit was compared to results obtained from LABScreen Single Antigen HLA Class II – Group 1, Cat #LS2A01, using data collected on a total of 512 samples. The comparison is based on the 63 antigens in common between the two products (Table 4B). The point estimates for overall agreement, PPA and NPA per antigen are depicted in Figure 2B.

Eplet analysis was done to further evaluate discordant results between the two methods and assess the performance of the antigens that are unique to the LIFECODES LSA Kits.

For discrepant analysis, eplet analysis was performed on the combined data from the LIFECODES LSA kits and the LABScreen kits. when an antigen was assigned positive for the LABScreen result and assigned negative for the LIFECODES LSA result and there was no eplet to explain why the antigen should be positive, the LABScreen result was changed to negative. When an antigen was assigned negative for the LABScreen result and assigned positive for the LIFECODES LSA result and there was an eplet to explain why the antigen should be positive, then the LABScreen result was changed to positive. The Class I results and Class II results after applying this correction are provided in Table 5A and 5B, respectively. The point estimates for overall agreement, PPA and NPA per antigen are depicted in Figures 3A and 3B for Class I and Class II, respecitively.

For beads unique to LIFECODES LSA, Eplet analysis was used to assign antigens as positive or negative as there was no comparator reagent available. These assignments were compared to the positive or negative results obtained with LIFECODES LSA. When using the eplet approach to assess the performance of the antigens unique to LIFECODES LSA kits, for Class I, the PPA was 77.43% (75.29%) and the NPA was 97.37% (96.97%), and for Class II, the PPA was 74.57% (73.17%) and the NPA was 96.89% (96.64%).

Table 4A. Initial Comparison

Combined (Three Sites)		LABScreen Single Antigen HLA Class I-Combi			% Agreement	93.81%
		Positive	Negative	Total	Concordance (95% Lower CI)	93.61%
LSA I	Positive	7504	685	8189	PPA (Point Estimate)	79.35%
	Negative	1953	32446	34399	PPA (95% Lower CI)	78.65%
	Total	9457	33131	42588	NPA (Point Estimate)	97.93%
					NPA (95% Lower CI)	97.78%

Figure 2A. Class I Clinical Comparison at Antigen Level

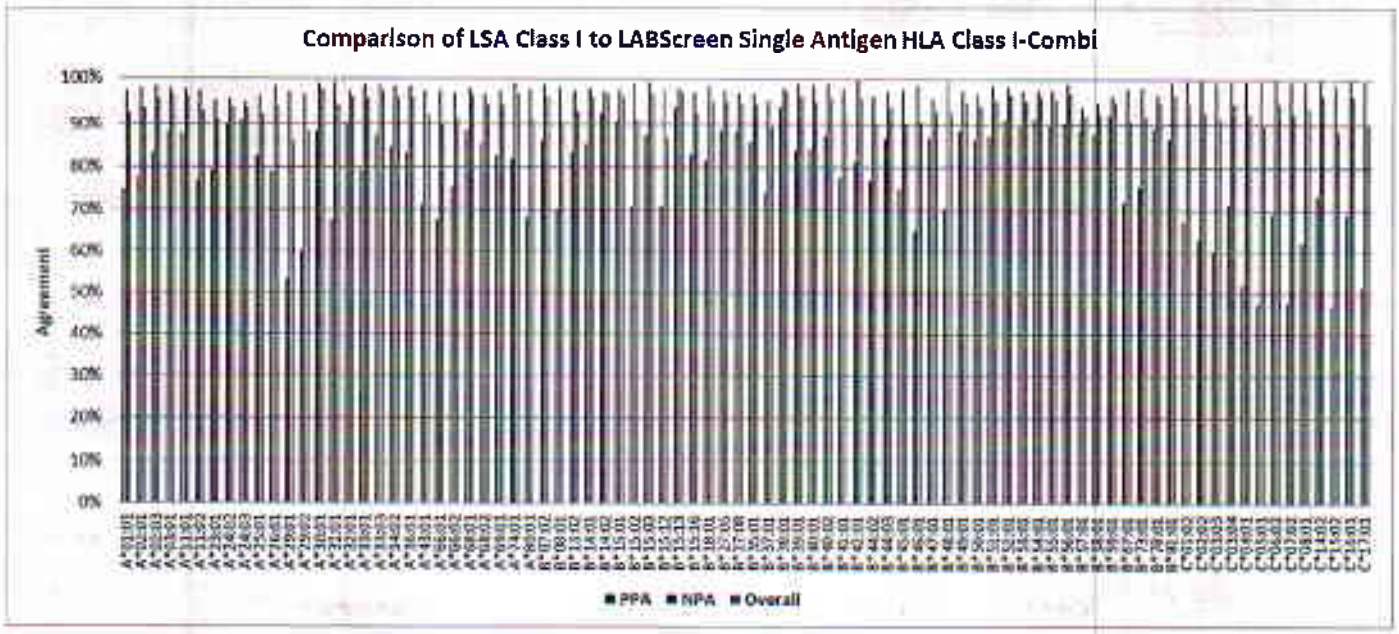


Table 4B. Initial Comparison

Combined (Three Sites)		LABScreen Single Antigen HLA Class II-Group 1			% Agreement	91.20%
		Positive	Negative	Total	Concordance (95% Lower CI)	90.94%
LSA II	Positive	4345	993	5338	PPA (Point Estimate)	70.18%
	Negative	1846	25072	26918	PPA (95% Lower CI)	69.21%
	Total	6191	26065	32256	NPA (Point Estimate)	96.19%
					NPA (95% Lower CI)	95.90%

Figure 2B. Class II Clinical Comparison at Antigen Level

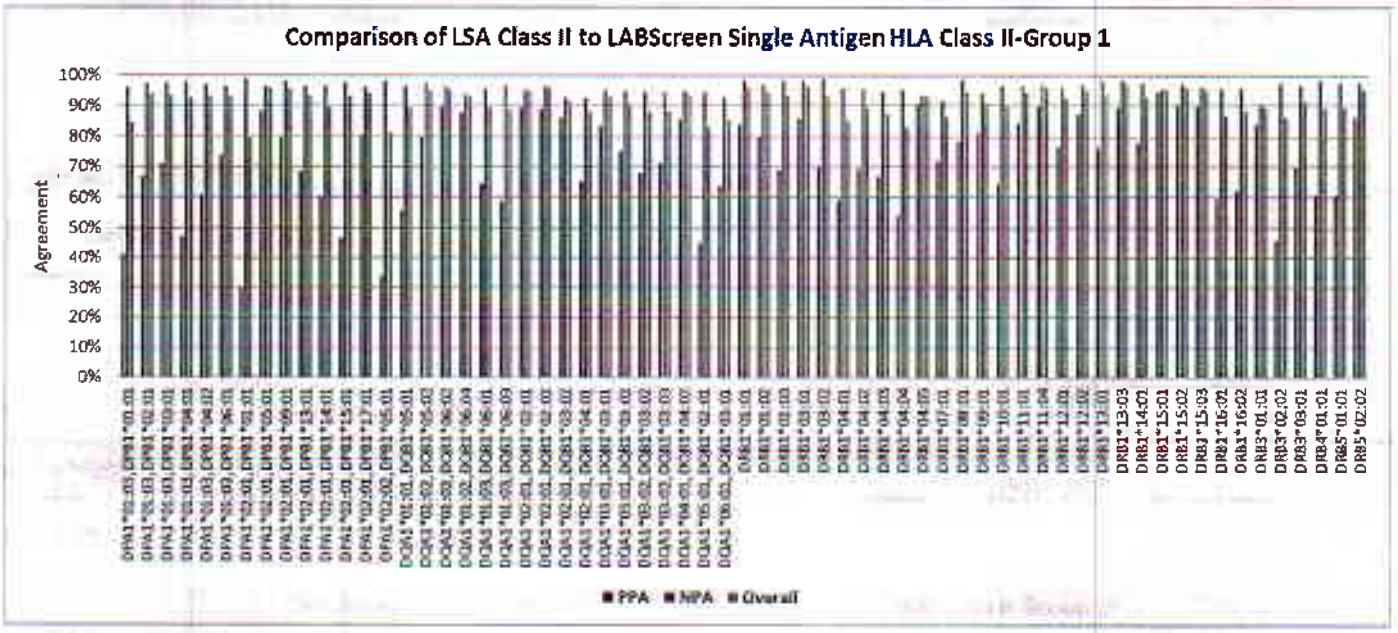


Table 5A. Comparison after Eplet Resolution*

Combined (Three Sites)		LABScreen Single Antigen HLA Class I-Combi (Eplet Corrected)			% Agreement	97.53%
		Positive	Negative	Total	Concordance (95% Lower CI)	97.40%
LSA I	Positive	7868	321	8189	PPA (Point Estimate)	91.48%
	Negative	733	33666	34399	PPA (95% Lower CI)	90.97%
	Total	8601	33987	42588	NPA (Point Estimate)	99.06%
					NPA (95% Lower CI)	98.97%

*The HLA Eplet Registry is for Research Purposes Only

Figure 3A. Class I Clinical Comparison at Antigen Level After Eplet Resolution

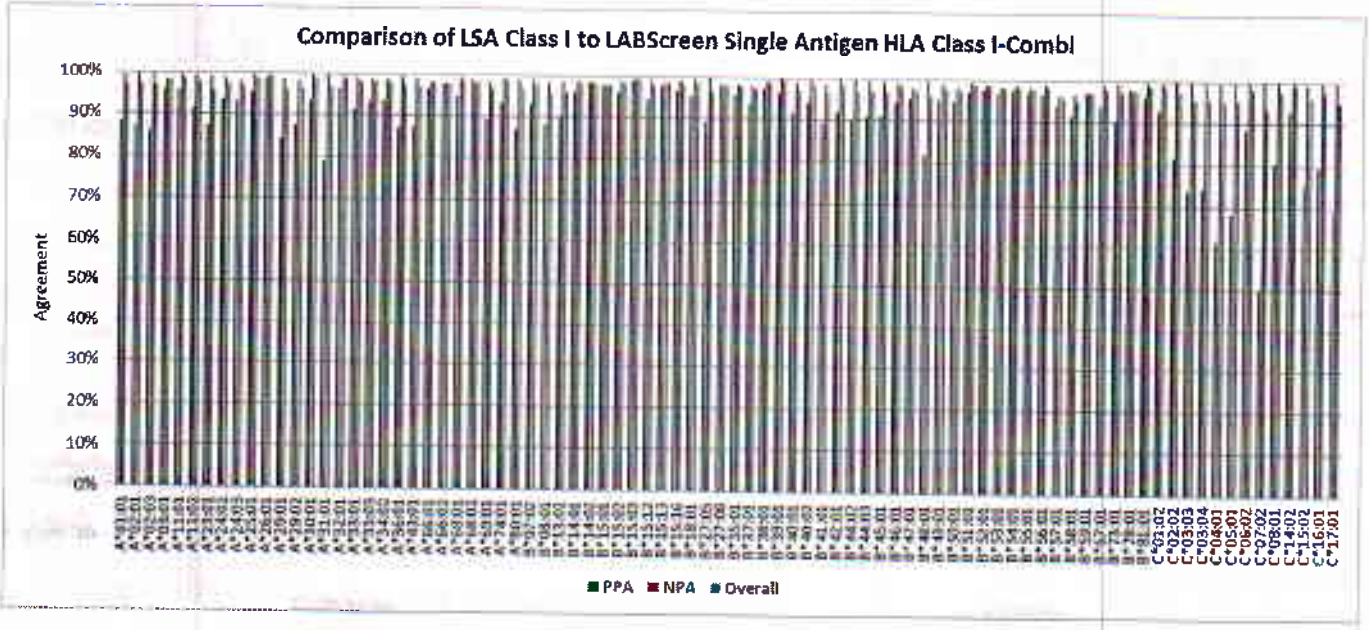
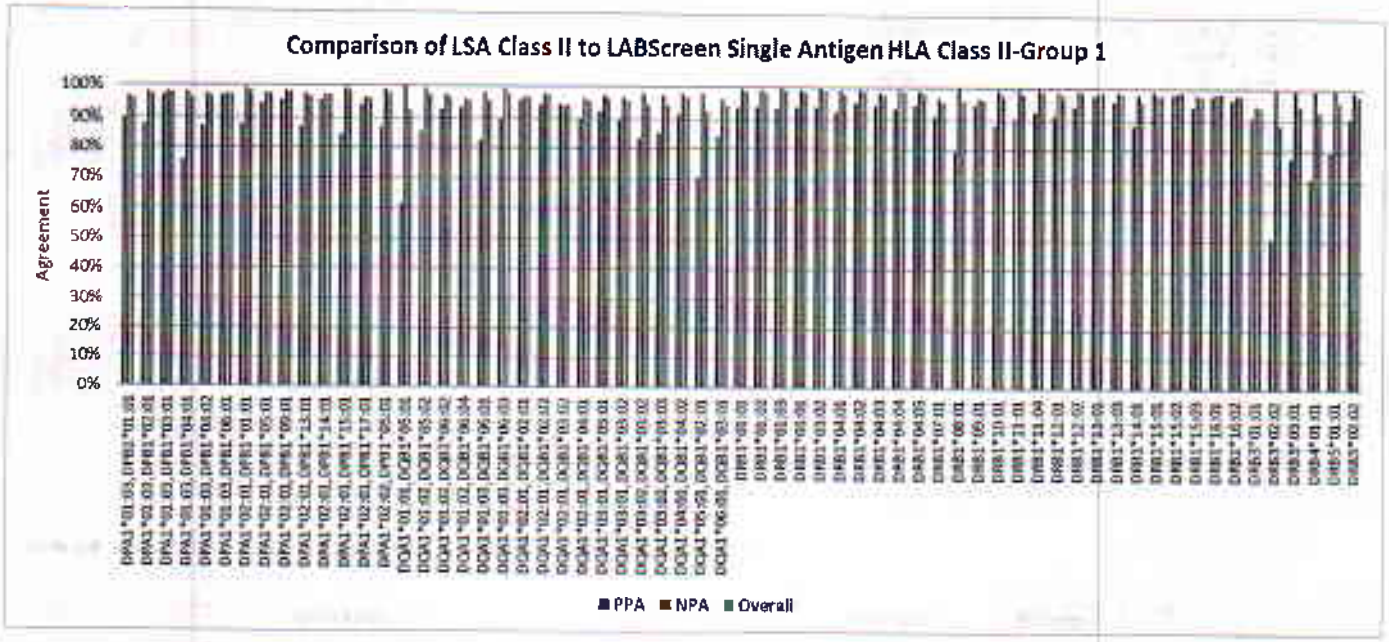


Table 5B. Comparison after Eplet Resolution*

Combined (Three Sites)		LABScreen Single Antigen HLA Class II-Group 1 (Eplet Corrected)			% Agreement	96.53%
		Positive	Negative	Total	Concordance (95% Lower CI)	96.36%
LSA II	Positive	4827	511	5338	PPA (Point Estimate)	88.80%
	Negative	609	26309	26918	PPA (95% Lower CI)	88.07%
	Total	5436	26820	32256	NPA (Point Estimate)	98.10%
					NPA (95% Lower CI)	97.95%

*The HLA Eplet Registry is for Research Purposes Only

Figure 3B. Class II Clinical Comparison at Antigen level After Eplet Resolution



Instrument Comparison

One hundred twenty four (124) samples were tested in triplicate on the Luminex 200 instrument to generate a consensus result for each antigen in the LSA1 or LSA2 products. The samples were run on 3 different Luminex FLEXMAP 3D instruments. The results from each FLEXMAP 3D were compared to the Luminex 200 consensus result. The average positive percent agreement and negative percent agreement were determined.

LSA Class I
The average positive percent agreement between the Luminex 200 and the Luminex FLEXMAP 3D instruments was 99.1% and the average negative percent agreement was 98.3%.

LSA Class II
The average positive percent agreement between the Luminex 200 and the Luminex FLEXMAP 3D instruments was 99.4% and the average negative percent agreement was 98.2%.

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TRADEMARKS USED

Luminex®	Luminex corporation	LIFECODES®	Immucor, Inc.
MATCH IT! ®	Immucor, Inc.	LABScreen™	Thermo Fisher Scientific