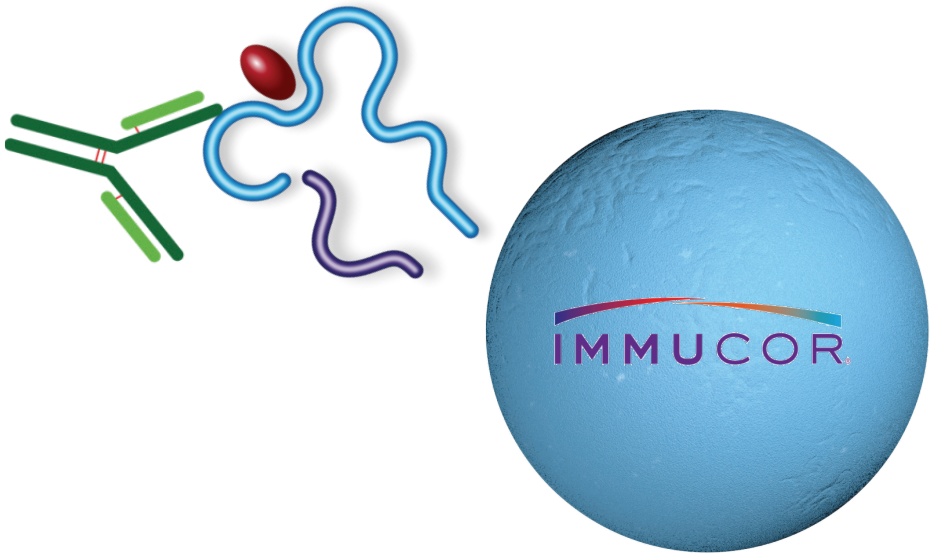
LIFECODES® Single Antigen Assay: Lowering the Denatured Antigen Barrier



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Introduction

While Single Antigen Testing becomes an increasingly important test to determine the presence of unacceptable antigens pre-transplant and to determine the necessity of desensitization treatment post-transplant, false positive reactivity due to the presence of denatured HLA antigens on the beads presents a well characterized clinical challenge[i]. There are four possible forms of HLA Class I (HLA-I) structural variants that can be present on Single Antigen Beads. The "native" HLA-I trimer (Figure 1) is found within natural cells whereas the other three structural variants, otherwise described as "denatured antigens" (Figure 2), are bi-products of the Single Antigen Bead manufacturing process[ii].

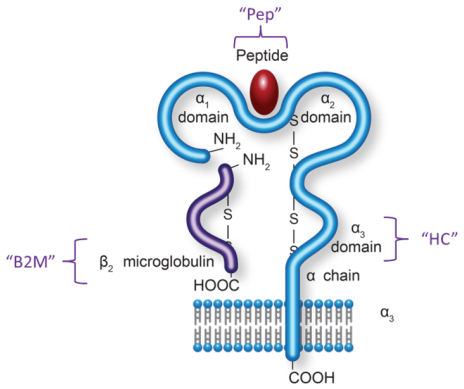

Figure 1 Native HLA trimer consists of an Alpha or "Heavy" Chain, Beta 2 microglobulin, and a peptide.

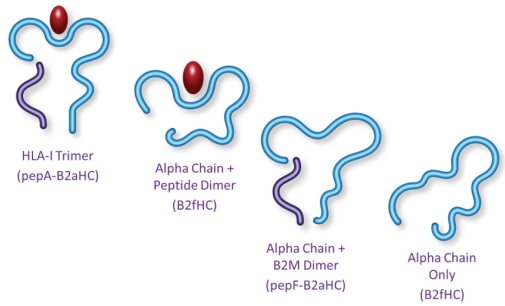

Figure 2 HLA-I trimer can dissociate into three structural variants: Alpha Chain + Peptide Dimer (β2fHC), Alpha Chain + B2M Dimer (pepF- β2aHC), and Alpha Chain Only (β2fHC).

Of these four variants, only the native HLA-I trimer has been associated with a significantly lower graft survival rate. Non-native HLA-I structural variants contain altered epitopes that can react with non-clinically relevant HLA-I antibodies and cause false positive reactivity[iii iv]. When Single Antigen assays indicate Donor Specific Antibody (DSA) is present and the crossmatch is negative, many labs spend considerable resources trying to understand if the Single Antigen results are clinically relevant. If not clearly identified, false positive results may result in the "inappropriate assignment of unacceptable antigens during transplant listing" which may consequently result in a patient not receiving a transplant even though the donor is a compatible match[v]. Additionally, DSA monitoring is routinely performed to determine if immunosuppression should be increased or decreased post-transplant. False positives could result in treating patients with immunosuppressive therapy unnecessarily[vi].

A variety of studies have been performed to characterize the antigen composition on Single Antigen Beads (SAB) available from One Lambda and Immucor. These studies demonstrate how the antigen composition on the beads contributes to false positive results when running Single Antigen assays.

The recent findings by Ravindranath et al at the Terasaki Foundation Laboratory demonstrate the Immucor LIFECODES SAB bead sets carry exclusively the HLA-I trimer whereas the SAB bead sets from One Lambda, the LABScreen® and iBeads (discontinued), carry both the native HLA-I trimer as well as one or more non-native structural variants. Because the structural variants can interact with non-clinically relevant antibodies, they are more likely to cause false positive reactivity[ii]. The proprietary Immucor SAB manufacturing process minimizes the non-native structural variants on the LIFECODES beads and therefore is a more reliable SAB assay to assess unacceptable antigens prior to transplantation.

Methods

Ravindranath et al utilized three monoclonal antibodies (W6/32, HC-10 and TFL-006) that distinguish structural variants of HLA-I trimer to understand the composition of Immucor's LIFECODES Single Antigen assay and One Lambda's LABScreen and iBead assays.

- The monoclonal antibody (mAb) W6/32 (IgG2a) (One Lambda, Canoga Park, CA, USA) binds to β2aHC (pepA-β2aHC) and pepF-β2aHC.
- The mAb HC-10 (IgG2a) (Nordic MUbio, Susteren, Netherlands) binds to pepF-β2aHC and β2fHC.
- The mAb TFL-006 (IgG2a), developed by immunizing HLA-E Pepf-β2fHC, binds to the β2fHC conformation of all HLA-I[vii viii].

mAb	Specificity - Positive	Specificity - Negative
W6/32	pepA-β2aHC and pepF-β2aHC	β2fHC
HC-10	pepF-β2aHC and β2fHC	pepA-β2aHC
TFL-006	β2fHC	β2aHC (pepA-β2aHC and pepF-β2aHC)

Figure 3. Monoclonal antibody specificities to the HLA-I structural variants surveyed by Ravindranath et al.

The SAB assays from Immucor and One Lambda were performed as per the One Lambda LAB Screen assay protocol to minimize differences in MFI values. All mABs were titrated to 10 ug/mL and the MFI cutoff of 1,000 was used to determine positive results.

Results

The native HLA-I trimer is comprised of two chains and a peptide: alpha chain, beta 2 microglobulin (β2M) chain and a peptide. The native HLA-I trimer has a specific epitope that binds to clinically significant HLA-I DSA. Patients with DSA to the native HLA-I trimer have been shown to have a significantly lower graft survival rate when compared to patients with no DSA or antibodies to non-native HLA-I structural variants[vi ix].

When HLA-I recombinant proteins are manufactured for SAB assays, any combination of the two amino acid chains and peptide can result. The non-native HLA-I structural variants assume epitopes that react with non-clinically relevant antibodies and can lead to false positive reactivity (Figure 4).

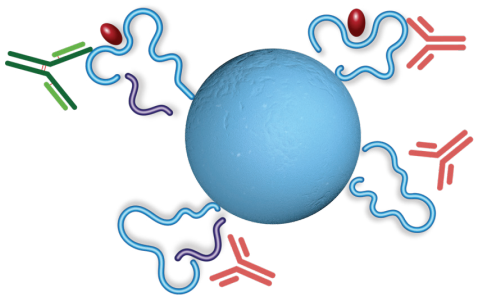

Figure 4 Native HLA-I trimer binds to clinically significant DSA whereas the HLA-I structural variants bind to non-clinically significant antibodies that can be misinterpreted as DSA.

In this study, Ravindranath et al used three monoclonal antibodies (mAb's) that react with different HLA-I antigen structural variances to detect which HLA-I variants were present on three Single Antigen Bead sets: LABScreen (One Lambda), iBeads (One Lambda) and LIFECODES Single Antigen Assay (Immucor).

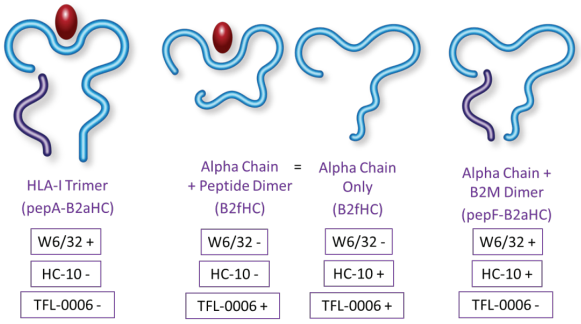

Figure 5 Monoclonal antibody reactivity patterns indicative of the presence of each HLA-I structural variant.

mAB W6/32

Figure 6 summarizes the median MFI of each SAB assay when hybridized with mAb W6/32. The positive reaction with mAb W6/32 across all loci shows all three SAB assays contain the clinically relevant native HLA-I trimer. The stronger signal of the LABScreen and iBeads is due to the mAB W6/32 reaction with the structural variant that does not include the peptide as demonstrated by their reactivity with mAb HC-10 outlined below.

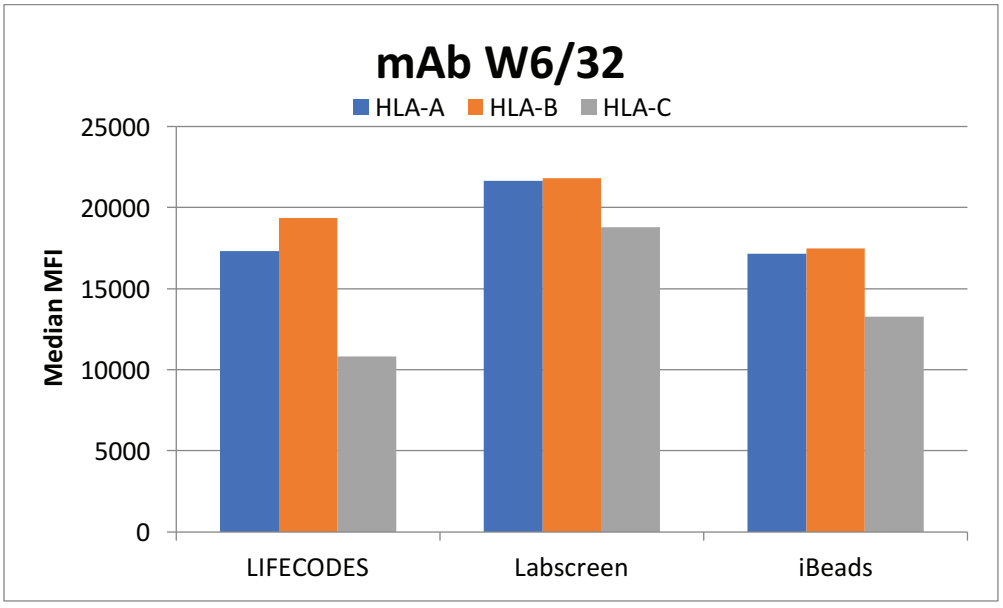

Figure 6 Representation of the data generated by Ravindranath et al demonstrating that all three bead sets contain the native HLA-I trimer. The higher signals with the One Lambda LABScreen beads may be due to the presence of the peptide free structural variant.

mAB HC-10

Figure 7 summarizes the median MFI of each SAB assay when hybridized with mAb HC-10. mAB HC-10 binds to the peptide free HLA-I structural variants: Alpha Chain Only (B2fHC) and Alpha Chain + B2M Dimer (pepF-B2aHC). Although there is some mAb HC-10 signal with the Immucor LIFECODES beads, the signal is lower than the established positive threshold of <1,000. The One Lambda iBeads signal indicates a presence of the HLA-I structural variants on the beads with signal high enough to be called positive using the 1,000 MFI cutoff.

In contrast, the very high signal on the One Lambda LifeScreen beads indicates a very large amount of Alpha Chain only and Alpha Chain + B2M dimer presence on the beads. Ravindranath et al suspect the high reactivity of the One Lambda LifeScreen beads with mAb W6/32 is due to the presence of the Alpha Chain + B2M Dimer and not purely to an increased density of the native HLA-I trimer on the beads as was suggested by Hiton and Parham.

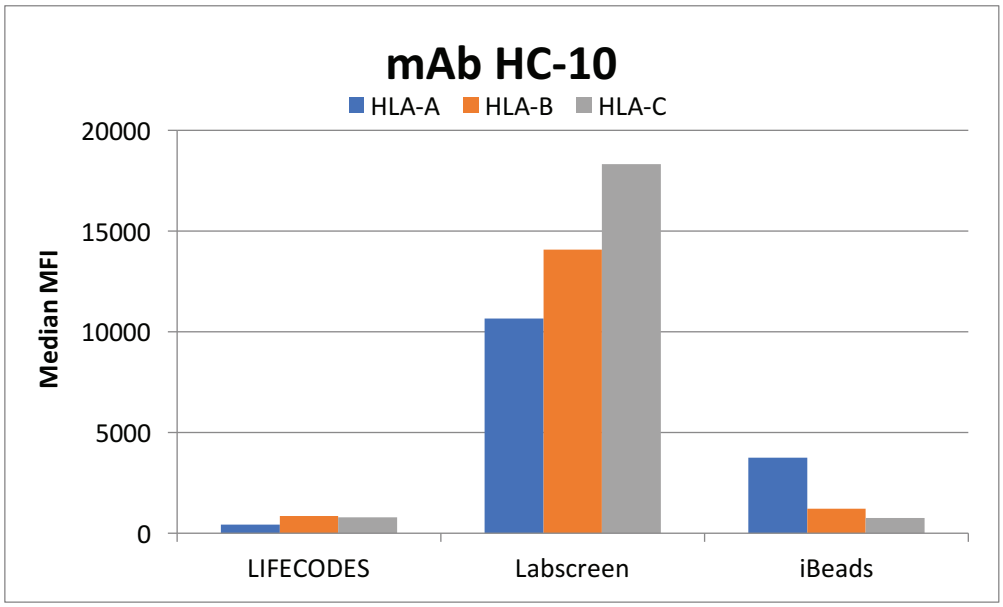

Figure 7 Representation of the data generated by Ravindranath et al demonstrating that only the Immucor LIFECODES beads are negative (<1,000 MFI) for two HLA-I non-clinically relevant structural variants. The One Lambda iBeads demonstrate low positivity, while the One Lambda LABScreen beads show very high positivity to the clinically non-relevant HLA-I structural variants.

mAB TFL-0006

Figure 8 shows the reactivity of the Single Antigen beads with mAb TFL-0006 which binds to the Alpha chain only when beta 2 microglobulin is absent (B2fHC). mAb TFL-0006 reactivity is indicative of either the Alpha Chain with the Peptide or the Alpha Chain without the Peptide. Strong reactivity was observed with One Lambda's LABScreen and low reactivity was observed with One Lambda's iBeads. The LIFECODES Single Antigen beads showed no reactivity with mAb TFL-006 indicating neither of the B2fHC variants are present on the beads.

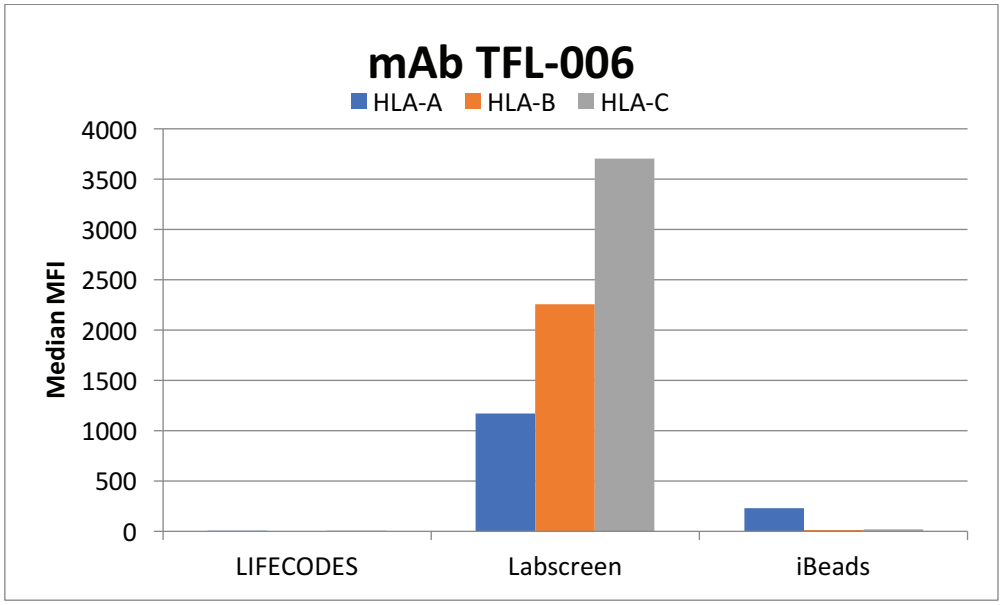

Figure 8 Representation of the data generated by Ravindranath et al demonstrating that only both Immucor LIFECODES SAB and One Lambda iBeads are negative (<1,000 MFI) for the two B2fHC structural variants, whereas the One Lambda LABScreen beads are positive, in particular for HLA-C.

Based on the mAb binding patterns, Ravindranath et al concluded that the One Lambda LABScreen beads contain both the native HLA-I trimer and all three structural variants, the One Lambda iBeads (discontinued) contained the native HLA-I trimer along with both the peptide free and peptide associated B2fHC structural variants, whereas only the Immucor LIFECODES Single Antigen beads carry the native HLA-I trimer "exclusively" and are therefore more useful for monitoring of the clinically relevant HLA-I DSAs.

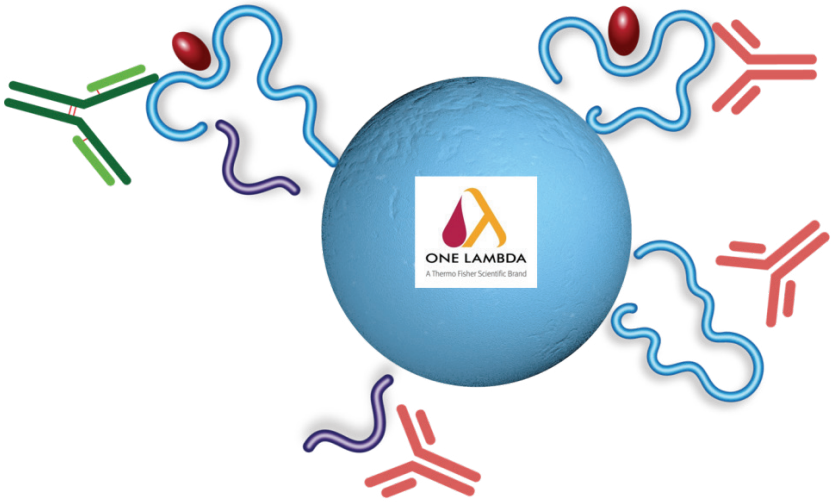

Figure 9 The One Lambda LABScreen beads contain the clinically relevant, native HLA-I trimer in addition to three structural variants that can bind to non-clinically relevant DSA.

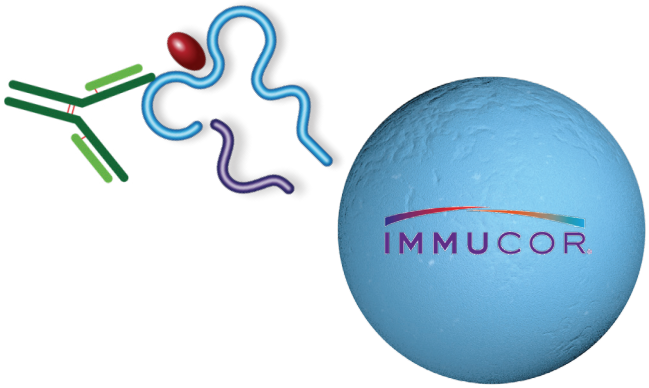

Figure 10 The Immucor LIFECODES Single Antigen beads carry "exclusively" the clinically relevant, native HLA-I trimer.

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Conclusion

Ravindranath et al have demonstrated that the Immucor LIFECODES Single Antigen Beads contain only the clinically relevant HLA-I trimer, unlike the SAB bead sets from One Lambda's LABScreen and iBeads, which contain both the HLA-I trimer as well non-clinically relevant structural variants.

The One Lambda LABScreen beads contain non-native HLA-I structural variants, otherwise known as "denatured antigens" which can bind to non-clinically relevant antibodies; therefore using the LABScreen kit for DSA screening is more likely to produce false positive results. False positives may result in the "inappropriate assignment of unacceptable antigens during transplant listing[v] " which may result in a patient not receiving a transplant even though the donor is a compatible match. Therefore using the Immucor LIFECODES Single Antigen Bead assay which contains exclusively the native HLA-I trimer, HLA laboratories can avoid unnecessary false positivity and potentially facilitate more patients receiving an organ transplant.

References

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[x]Hilton H.G. and Parham P., Direct binding to antigen-coated beads refines the specificity and cross-reactivity of four monoclonal antibodies that recognize polymorphic epitopes of HLA class I molecules, Tissue Antigens 81, 2013, 212–220.

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