Elevating Precision In HLA Antibody Identification: A Study of the NEXA Single Antigen Extended Panel in Transplant Immunology

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Aim

As HLA laboratories increasingly adopt high-resolution typing for solid organ transplant patients, there is a growing need for antibody detection methods that offer broader allele coverage. To address this clinical need, Werfen developed the NEXA Single Antigen Extended Panel, which expands class I allele representation from ~100 alleles in the current LSA1 Single Antigen Bead (SAB) assay to over 150 specificities. The goal of this study is to evaluate the performance and limitations of the NEXA Single Antigen Extended Panel and to assess the concordance in antibody detection in comparison to the LSA1 panel. Additionally, the study aims to explore the utility of the expanded panel in the interpretation and assignment of HLA antibodies.

Methods

Thirty-eight serum samples were thawed and processed for IgG purification using the Zeba™ Spin Desalting Plate followed by the Melon™ Gel IgG Spin Purification Kit to isolate IgG antibodies. The sera were then run in parallel using the LSA1 SAB kit and the NEXA Single Antigen Extended Panel class I kit on the Luminex™ FlexMap 3D platform. Mean Fluorescence intensity (MFI) values were collected (correction factor was not applied) and antibody patterns were analyzed.

Figure 1. Behavior of sera tested by LSA and class I NEXA panels

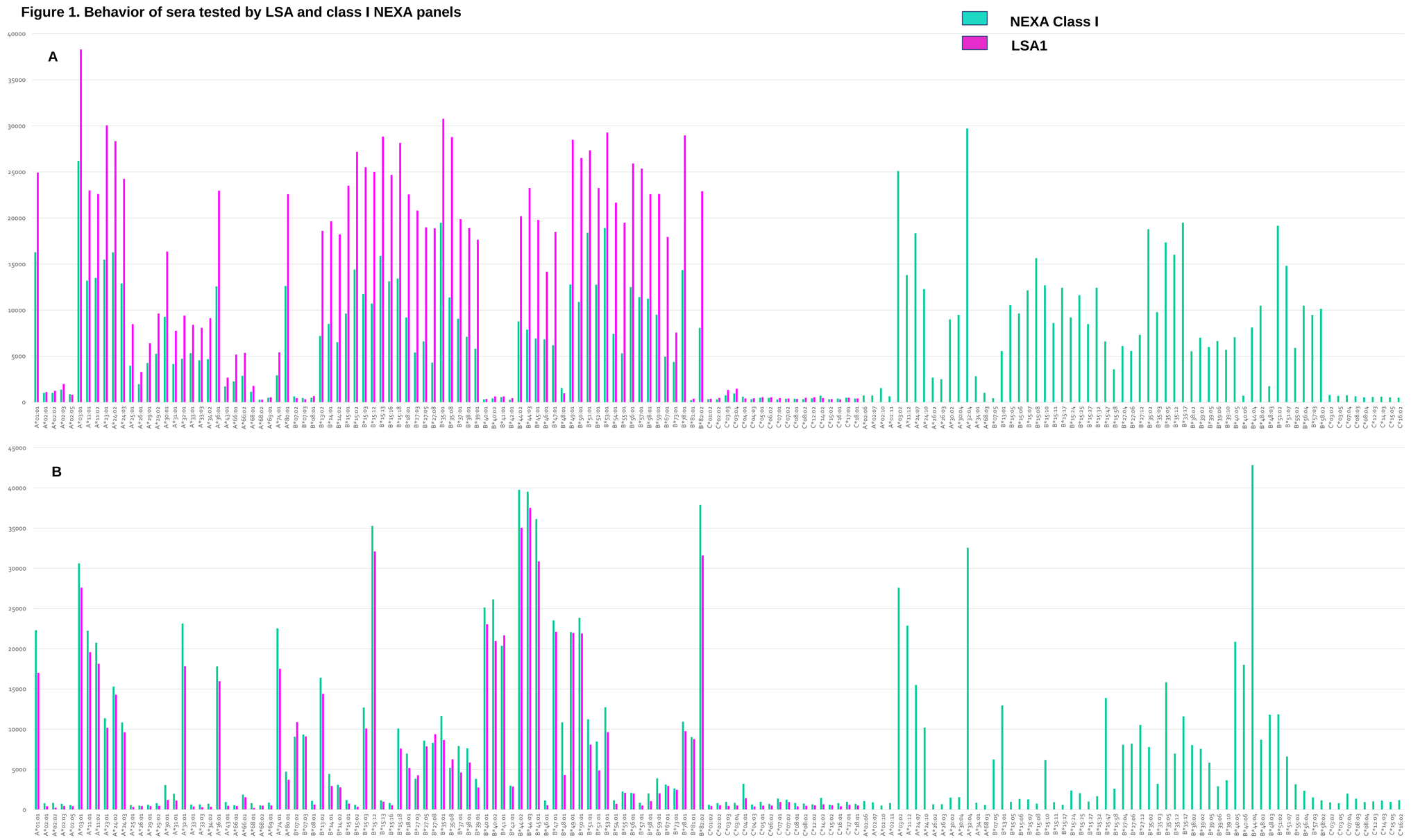
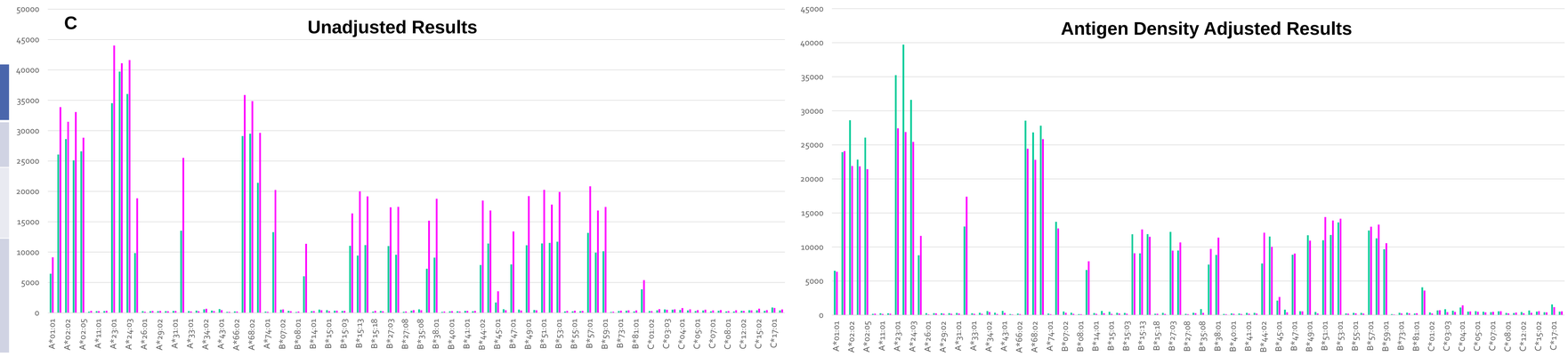


Table 1. A comparison between LSA1 and NEXA class I panels

	LSA1 (96 alleles)			NEXA class I (159 alleles)		
Locus	A	B	C	A	B	C
Panel composition	30 alleles	48 alleles	18 alleles	30 + 15 alleles	48 + 39 alleles	18 + 9 alleles
Relative Antigen Density	High: 1.911, B27:08 Low: 0.55, C*04:01 Average: 1.44 Luminex bead diameter: 5.6µ			High: 1.18, A*11:02 Low: 0.2, C*07:04 Average: 0.91 Luminex bead diameter: 6.5µ (16% larger)		



Results

A total of 38 patient sera were tested. Of those, 19 sera had higher MFI with LSA (pink) as compared with NEXA (turquoise) (Example: **Figure 1A**), 9 sera showed comparable LSA and NEXA MFI (not shown), and 10 sera had higher MFI with NEXA compared with LSA (Example: **Figure 1B**). **Figure 1C** shows a serum with higher MFI on LSA, however, when the MFI values were adjusted for the relative antigen density difference between the LSA and NEXA beads (see **Table 1**), LSA and NEXA MFI were comparable.

Overall, a good correlation was observed between LSA and NEXA for antibody specificities included in both panels. The inclusion of additional alleles in the extended panel enhanced antibody analysis by providing more information on reactivity patterns, which contributed to a better understanding of the patient's antibody profile and to an improved discrimination between specific and non-specific antibodies.

Specific alleles that assisted in identification of certain epitopes in the tested sera included A*02:10, A*24:10, A*26:03 (76VDT); A*32:04 (144K, 149AH); A*30:04 (151H); B*15:17 (113Y); when used together, B*48:02 and B*15:47, helped in the assignment of 163L and 163E in two different sera.

Conclusions

The NEXA Single Antigen Extended Panel enables enhanced antibody analysis and interpretation, making it especially valuable for highly sensitized patients. Its ability to provide additional information compared to the standard antibody panel has the potential to improve patient care and outcomes, and to guide clinical decision making, particularly in complex transplant scenarios. Although, some sera exhibited lower MFI values with NEXA compared with LSA1, when the MFI values were adjusted for the relative antigen density the results were comparable.

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