

Breaking Down Pan-C Using One Lambda™ MagSort™

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Introduction

Pan-C cases have been previously observed with speculation that reactivity was due to denatured antigen. The Pan-C sample in this study, although negative for C*07:02, was tested using the LABScreen™ Single Antigen HLA Class I (LS1A04) and acid-treated LS1A04 beads. The sample was also tested on another bead-based antibody detection assay for confirmation. In addition, the sample was adsorbed using three MagSort™ bead products (C*01:02, C*07:02, and C*17:01) and the eluate tested against the SA panel.

Materials and methods

LABScreen Single Antigen HLA Class I (Catalog ID: LS1A04) Lot 15
 Serum 3 04/2023 obtained from UK NEQAS
 MagSort Class I HLA-C:
 MagSort C0102 (Catalog ID: MAG1C0102)
 MagSort C0702 (Catalog ID: MAG1C0702)
 MagSort C1701 (Catalog ID: MAG1C1701)

Test Method(s)

LABScreen product insert
 MagSort Application Note
 Acid treatment: LS1A04 incubated in an acid solution for 1 hour before testing

Results

Untreated serum was positive for all HLA-C alleles except for C*07:02 in both bead-based products (Fig. 1). Acid treatment lowered reactivity suggesting that the antibody is not against denatured antigen (Fig. 2). Eluted antibody from the C*01:02 and C*17:01 MagSort beads correlate to the 184H and 80K eplets, respectively (Fig. 3). Elution from the C*07:02 MagSort bead was negative. The data shows that this Pan-C serum may contain two overlapping antibody specificities, and not a single Pan-C. This data suggests that the observed reactivity is genuine and should not be omitted as false reactivity.

Results

Figures & Tables

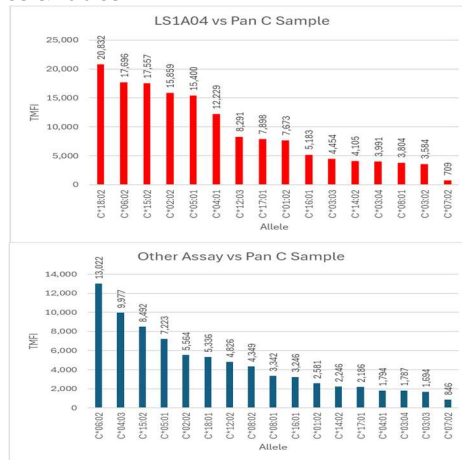


Figure 1. LS1A04 (red) and other assay (blue) histogram of C alleles sorted by TMFI strength. In general, there is some agreement between which alleles are strongest reacting. Both assays have C*07:02 as the lowest/negative reaction. Notable strength differences between the two assays is observed

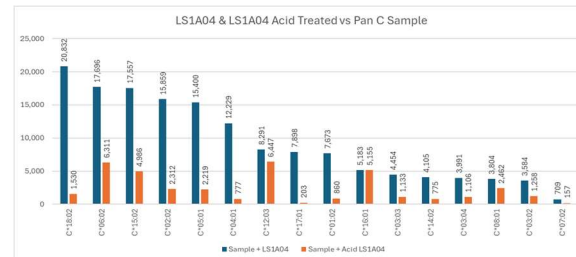


Figure 2. TMFI values of HLA-C alleles on LS1A04 and acid-treated LS1A04 when tested against the pan C sample. The acid treatment can denature Class I antigens by dissociating the beta-2-microglobulin (β₂M). Reacting serum with acid-treated beads may indicate whether the antibody is against denatured antigen. In this case, the reactivity does not increase when tested with the acid-treated beads suggesting that the antibodies are not against denatured antigen.

Results

Figures & Tables



Figure 3. MagSort eluate results. When the Pan-C sample was adsorbed and eluted with C*01:02, the reactivity pattern corresponded to 184H. When adsorbed and eluted with C*07:02 no antibody was isolated. When adsorbed and eluted with C*17:01, the reactivity pattern corresponded to 80K.

Eplet ID	Eplet Name	Alleles Detected by Luminex Assays
95	80K	C*02:02, C*02:10, C*04:01, C*04:03, C*05:01, C*06:02, C*15:02, C*15:05, C*16:02, C*17:01, C*17:03, C*18:01, C*18:02
202	184H	C*01:02, C*01:03, C*02:02, C*02:10, C*03:02, C*03:03, C*03:04, C*04:01, C*04:03, C*05:01, C*06:02, C*08:01, C*08:02, C*08:03, C*08:04, C*12:02, C*12:03, C*14:02, C*14:03, C*15:02, C*15:05, C*16:01, C*16:02, C*18:01, C*18:02

Table 1. Associated eplets in case-study sample from Eplet Registry

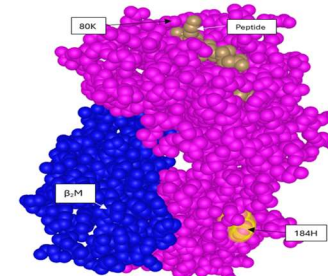


Figure 4. Crystal structure of HLA-C pointing to positions 80K, 184H, peptide and β₂M.

Conclusions

Genuine reactivities were observed following adsorption and elution using specific antigen coated MagSort beads. Assays from two different vendors were able to confirm the HLA-C reactivity, with the One Lambda product having a higher degree of sensitivity. Reduced reactivity to acid treated beads demonstrated antibody to intact HLA was observed. The sensitivity of LABScreen Single Antigen beads has sometimes led to questions of false reactivity, especially in multispecific samples, but MagSort beads may assist in researching the potential of overlapping, genuine antibody specificities.

References

- HLA Eplet Registry (<https://epregistry.com.br>)
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