Epitope Analysis of a Serum Sample from a Transplant Patient Using LABScreen™, MagSort™ and Cell-based Adsorption Elution Reveals Further Resolution of DQ antibody Reactivity

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Introduction

With polyclonal antibody samples (such as human serum), it can be difficult to resolve the epitope(s) involved in binding. By using tools like our DQ SAB panel, MagSort, and cell adsorption elution, we aimed for further resolution. The ability to resolve epitopes has many implications on the accurate assessment of immunogenicity.

HLA mismatch risk assessment is trending from antigen (Ag) to epitope-based assessment. A novel single antigen bead (SAB) panel focused on HLA-DQ was developed to facilitate this transition. The panel's utility has been demonstrated with a serum sample, S10016B, from a transplant patient who has undergone rejection. In this study, we aim to demonstrate a workflow that can be used to analyze serum samples to a higher resolution with tools like the DQ SAB panel, MagSort, and cell based adsorption elution[1].

Materials and methods

Sample Preparation

Serum samples of transplant patients who have undergone rejection episode(s) were purchased from BloodSource (Sacramento, California) and initially screened with LS2A01. One sample, S10016B, showed substantial reactivity to DQ antigens and was selected for further testing with MagSort as well as a cell based adsorption elution protocol. DQA1*05:01/ DQB1*02:01 was coated for the MagSort portion of the work. For the cell based adsorption elution, we transfected a host cell expressing no HLA class II antigens with DQA1*05:01/DQB1*02:03. 5 million cells of this recombinant cell line were used per adsorption elution. After Magsort and A/E, samples were run against LS2A01 and a DQ SAB panel. FM5148 and FM5203 are hybridoma clones that were generated in-house.

Test Method(s)

Flow cytometry was performed on the host cell line and recombinant cell line with 1 million total cells each. The cells were stained with FM5148 (a pan-DQ antibody). The data was acquired on the BD Accuri C6. The MagSort beads were run according to the product insert. For cell based adsorption elution, 200µLs of S10016B was incubated with 5 million recombinant cells exclusively expressing DQA1*05:01 /DQB1*02:03. LS2A01 and DQ SAB panel were run according to the product insert and the data was acquired on a Luminex FM3D.

Data Analysis

The data was analyzed using HLA Fusion 4.7. The new AminoAcid module was used to gain further insight on the epitope. pHLA3D was used to gain a visual understanding of the topography of the antigens.

Results

Both MagSort and cell based adsorption elution were able to further resolve the epitope in the case of S10016B. All of the DQ reactivity for S10016B could be explained by two different epitope scenarios. Either 40GR or the combination of 66IT and 75S could explain the entire DQ reactivity for sample S10016B. After MagSort and cell A/E, we were able to resolve the reactivity to 40GR by ruling out the combination of 66IT and 75S.

Figure 1. Flow cytometry data of the recombinant cell line expressing DQA1*05:01/DQB1*02:03 (in red) and the host cell (in black).

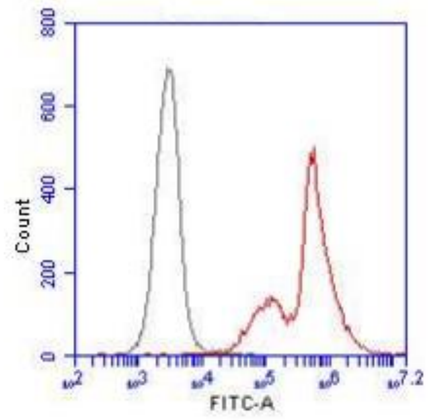
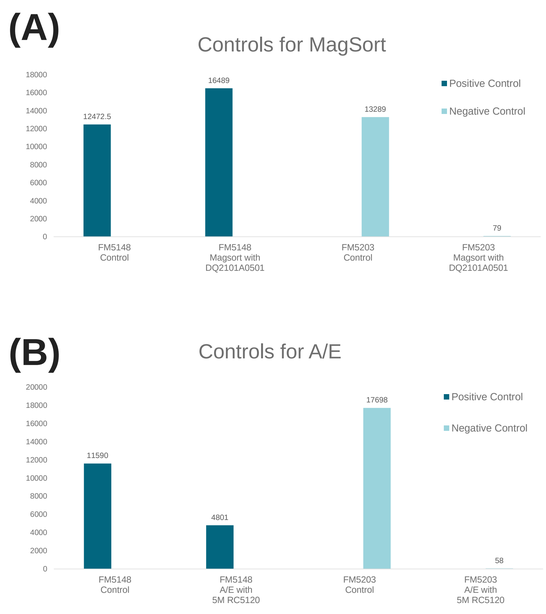


Figure 2. Amino Acid Module in Fusion 4.7 allows us to visualize the MFI with the sequence side-by-side.

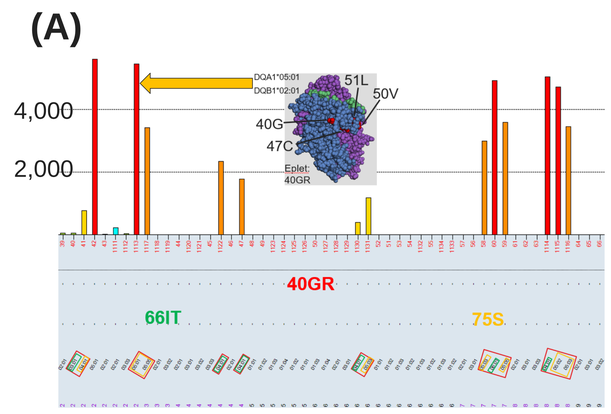
Allele	Pan	Posi	MFI	40	41	42	43	44	45	46	47	48	49	50	51
DQA1*05:01	☑	☑	5455	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*06:01	☑	☑	4919	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*05:02	☑	☑	4717	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*05:05	☑	☑	3422	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*05:03	☑	☐	1187	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*04:01	☑	☐	398	G	R	K	E	T	V	W	C	L	P	V	L
DQA1*01:01	☑	☐	0	E	R	K	E	T	A	W	R	W	P	E	F
DQA1*01:02	☑	☐	0	E	R	K	E	T	A	W	R	W	P	E	F
DQA1*01:03	☑	☐	0	E	K	K	E	T	A	W	R	W	P	E	F
DQA1*01:04	☑	☐	0	E	R	K	E	T	A	W	R	W	P	E	F
DQA1*02:01	☑	☐	0	E	R	K	E	T	V	W	K	L	P	L	F
DQA1*03:01	☑	☐	0	E	R	K	E	T	V	W	Q	L	P	L	F
DQA1*03:02	☑	☐	0	E	R	K	E	T	V	W	Q	L	P	L	F
DQA1*03:03	☑	☐	0	E	R	K	E	T	V	W	Q	L	P	L	F

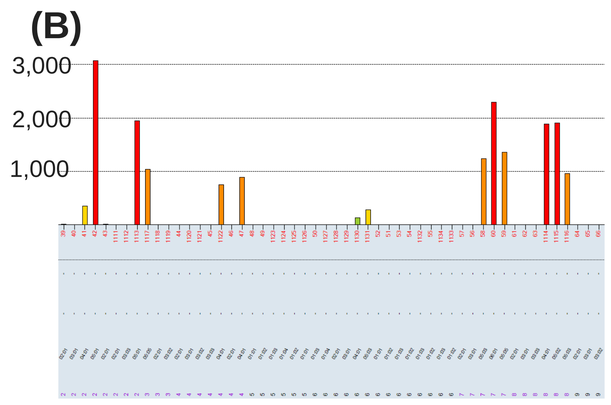
Figure 3. Positive and negative controls for (A) MagSort and (B) Cell Adsorption Elution.

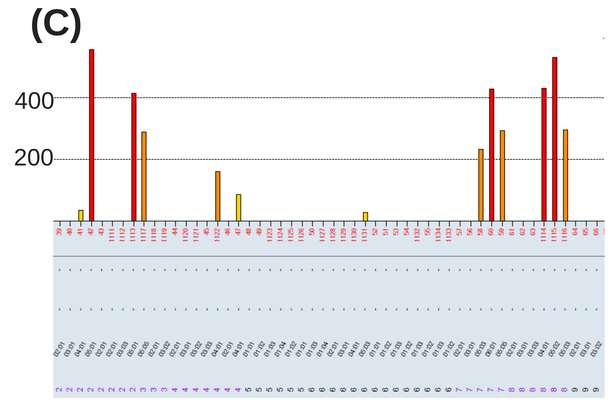


Results (Continued)

Figure 4. (A) The serum sample S10016B was run at neat against LS2A01 and the DQ SAB panel. (B) S10016B was adsorbed onto 5 million recombinant cells exclusively expressing DQA1*05:01/DQB1*02:03. After eluting, the sample was run against LS2A01 and the DQ SAB panel. (C) S10016B was adsorbed onto Magsort beads coated with DQA1*05:01/DQB1*02:01. The eluted product was then run against LS2A01 and the DQ SAB panel.







Conclusions

The recombinant cell line used for cell adsorption elution was shown to have 220x fold expression when compared to the host cell. Both MagSort and cell based adsorption elution protocol were able to add additional resolution to the binding between S10016B and various DQ antigens. Both tools were able to rule out the combination of 66IT and 75S when explaining the reactivity between S10016B and various DQ antigens. By further resolving this interaction, we gain insight to the immunogenicity of different epitopes. The ability to identify critical residues of an epitope has many implications on the future assessment of antigenicity.

References

1. Liwski RS, Tafulo S, Carroll R, Lan JH, Greenshields AL. Cutting through the weeds: Evaluation of a novel adsorption with crossmatch cells and elution protocol to sharpen HLA antibody identification by the single antigen bead assay. Front Genet. 2022 Nov 30;13:1059650. doi: 10.3389/fgene.2022.1059650. PMID: 36531234; PMCID: PMC9748275.
2. pHLA3D: An online database of predicted three-dimensional structures of HLA molecules.

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