Poster 201: Resolving Anti-HLA Specific Complexities Using Phenotype Beads: A Dual Assay Consideration

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Rationale

- Single antigen testing often shows well-characterized patterns of reactivity even in patients not exposed to HLA antigens which present important challenges for clinical interpretation
- It is critical to determine precise reactivity since erroneous specificities may falsely elevate the patient cPRA and potentially preclude transplantation without biological cause.

Objective

- To deconvolute single antigen reactivity patterns by comparing the **One Lambda LABScreen Single Antigen Bead (OLSAB)** assay (which uses recombinant protein bonded in a manufacturing process that may lead to denatured HLA molecules) with **Immucor LIFECODES ID beads (IMID)** kit (that employs EBV transformed cell lines expressing phenotypic protein

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Methods

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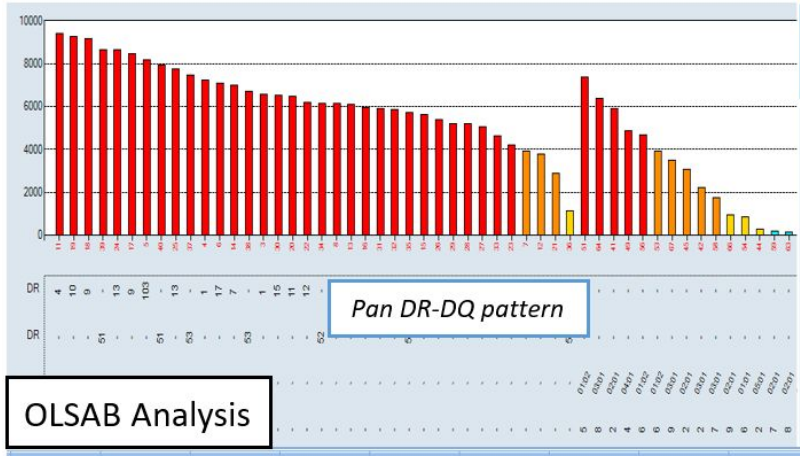
- Sera showing non-specific patterns of reactivity at HLA class I (n=16) and class II (n=20) on OLSAB were selected
- These were classified into the spurious patterns and analyzed using CREG groups and epitope analysis on MatchMaker
- All sera were tested again using the IMID kit and surrogate

Class I Patterns	Class II Patterns
• A80 or B76 alone • Cw*1, 12, 15 • High background reactivity • Pan C	• DPB1*1, 5, DR53 • Pan DR ± DQ • DQA1*01:03/DQB1 *06:03 • High background reactivity • DQA1*05:01/DQB1 *02:01

Allele	Panel	Pos	MFI	248M	1C 27% 3/11	184H 20% 3/15	76VS 20% 2/10	267PE 20% 3/15	21H 20% 1/5	69RT 18% 2/11
C*01:02	☑	☑	6502	+	/	/	/	/		/
C*12:03	☑	☑	5003		/	/	/	/		
C*15:02	☑	☑	2558		/	/		/	/	/
A*68:02	☑	☐	695							
C*03:03	☑	☐	691			/	/	/	/	/
C*03:04	☑	☐	450			/	/	/	/	/
C*03:02	☑	☐	322			/	/	/	/	/
B*15:12	☑	☐	286							
B*15:16	☑	☐	284							

Cw1 12 15 pattern

HLA Fusion MatchMaker Analysis



OLSAB Analysis

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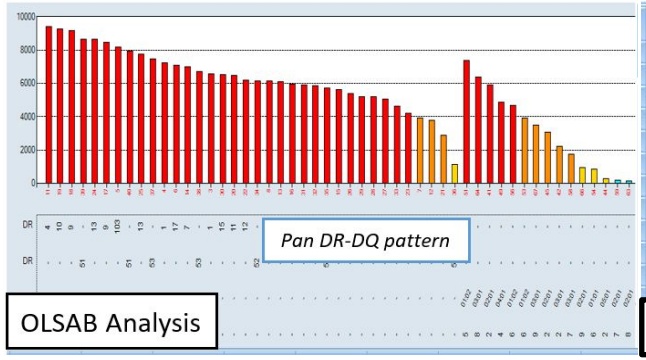
Results & Conclusions

Results

- IMID MatchIt software Tail End analysis often showed 0% PRA and no particular antigen groupings with low MFI reactivity
- The IMID kit includes additional DQA specificities to confirm or rule out questionable reactivity



OLSAB Analysis

Raw Value	Adjust 1	Adjust 2	Adjust 3	Adjust C	Assignment	DR1	DR2	DR5x1	DR5x2	DQA1	DQA2	DQB1	DQB2
438	-2.12	0.44	-0.52	-4.05	Negative					DQA1*05:01		DQB1*02:01	
229	-2.86	-1.25	-1.84	-4.6	Negative					DQA1*05:05		DQB1*03:01	
199	-2.86	-1.86	-1.63	-4.99	Negative	DRB1*04:05	DRB1*15:03	DRB4*01:03	DRB5*01:0	DQA1*01:02	DQA1*03:03	DQB1*03:02	DQB1*06:02
144	-3.23	-1.96	-2.46	-6.07	Negative	DRB1*15:03		DRB5*01:01		DQA1*01:02		DQB1*06:02	
143	-3.2	-2.13	-1.97	-6.0	Negative	DRB1*09:01	DRB1*14:01	DRB3*02:02	DRB4*01:0	DQA1*01:05	DQA1*03:02	DQB1*03:03	DQB1*05:03
138	-3.24	-2.3	-2.1	-6.17	Negative					DQA1*02:01	DQA1*05:01	DQB1*02:01	DQB1*02:02
138	-3.24	-1.59	-2.15	-5.82	Negative					DQA1*03:01		DQB1*03:02	
137	-3.14	-1.69	-2.15	-6.03	Negative					DQA1*04:01	DQA1*05:05	DQB1*03:19	DQB1*04:02
132	-2.9	-1.74	-1.51	-5.22	Negative	DRB1*01:01	DRB1*04:01	DRB4*01:01		DQA1*01:01	DQA1*03:03	DQB1*03:01	DQB1*05:01
131	-2.99	-1.58	-1.65	-5.42	Negative	DRB1*01:03				DQA1*01:01		DQB1*05:01	
126	-3.51	-2.28	-2.42	-7.03	Negative					DQA1*03:02	DQA1*03:03	DQB1*03:03	DQB1*04:01
				-5.3	Negative	DRB1*01:03	DRB1*08:04			DQA1*01:01	DQA1*04:01	DQB1*04:02	DQB1*05:01
				-6.48	Negative					DQA1*03:02		DQB1*03:03	
				-5.69	Negative	DRB1*03:01	DRB1*11:01	DRB3*02:02		DQA1*05:01	DQA1*05:05	DQB1*02:01	DQB1*03:01

Negative Assignment

IMID MatchIT Analysis

Conclusion

IMID kits help to deconvolute many of the complex, non-specific patterns of reactivity seen on OLSAB and may better reveal biological interactions.

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