

## ABSTRACT

Virtual crossmatch (vXM) is widely used as the final immunological risk assessment prior to solid organ transplantation. Accurate antibodies identification is critical to appropriate donor selection, and misinterpretation of alpha vs beta-specific antibodies can severely impact vXM results.

## METHOD

Our lab routinely uses One Lambda LABScreen Single Antigen Assay (OL-SAB) and in certain cases LIFECODES Single Antigen Assay (LC-SAB). We reviewed a total of 1,714 patients whose sera has been tested by both OL-SAB and LC-SAB in our lab since 2019 to determine the patients that had antibodies to the alpha chain of the HLA-DQ protein which could not be resolved by the routine OL-SAB panel.

## CONCLUSIONS

Although one SAB platform can provide an accurate assessment of a patient’s antibodies profile in most cases, some patients may have ambiguous antibodies reactivity patterns which may result in misinterpretation and can potentially lead to transplantation with incompatible donors if not resolved.

These cases highlight the significant shortcoming of the limited SAB beads coverage that can impact epitope identification.

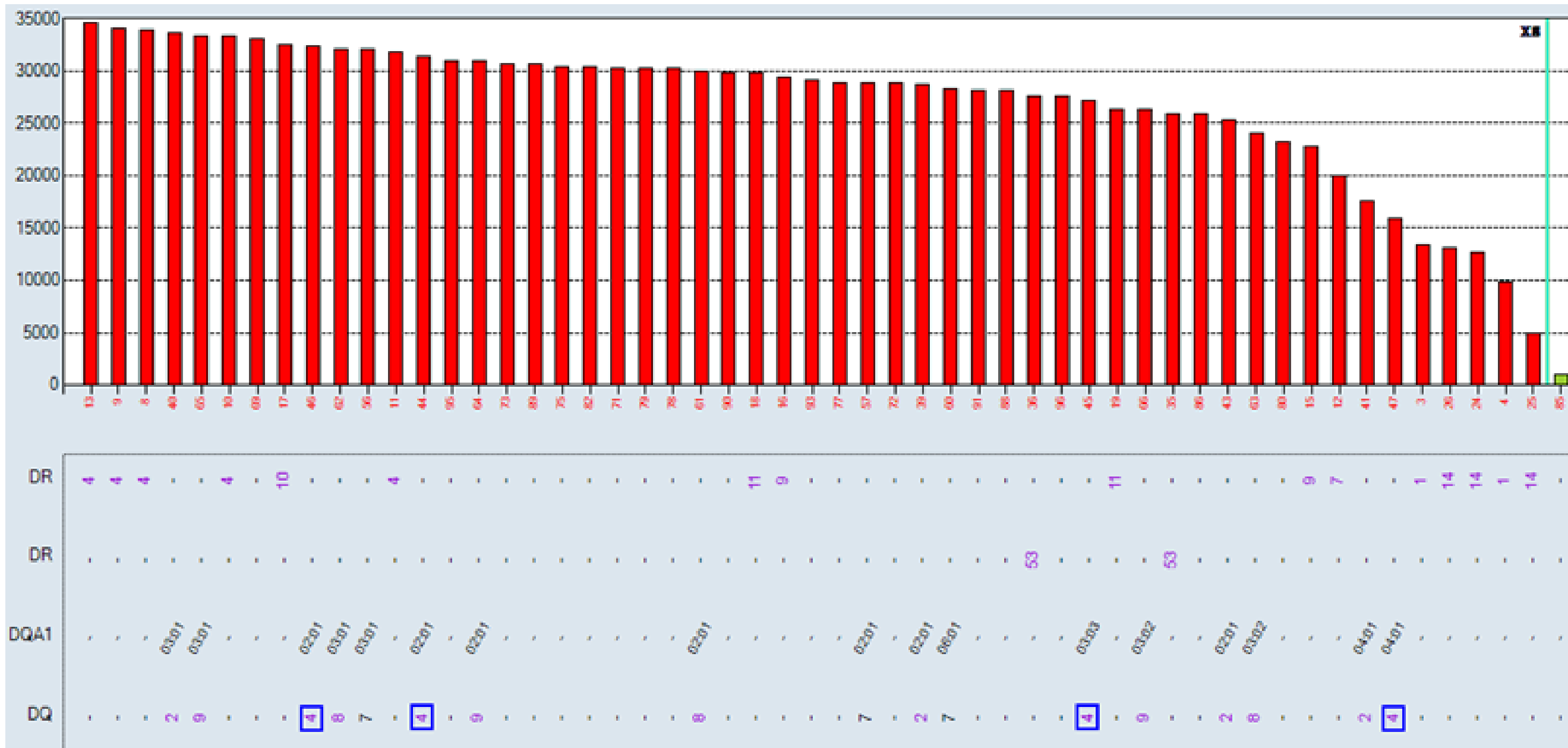
Case 1: A male patient with a prior kidney transplant was being evaluated for his second transplant. Based on the positive specificities, we determined that his class II antibodies were likely directed to the 84QL (DQ2/4/7/8/9) epitope (**Fig 1**).

Interestingly, LC-SAB demonstrated a single positive DQ6 bead (**Fig 2**).

LC-SAB contains one DQA1\*02:01-DQB1\*06:01 heterodimer bead, whereas OL-SAB DQ5/DQ6 beads only contain DQA1\*01 alleles. Thus, the class II Antibodies were not directed to the DQ beta proteins but rather to the 61FT4 epitope in the DQ alpha chains. Of 1,714 patients analyzed by LC-SAB since 2019, 16 (0.93%) were confirmed to have antibodies to the 61FT4 epitope. While DQ5 and DQ6 are largely associated with DQA1\*01 alleles, there are several populations in which DQ5 or DQ6 are associated with other DQA1 alleles.

**Table 1** summarizes those haplotypes found just within the United States<sup>1</sup>.

## Figure 3.



## Figure 4.

Assignment	BG Adjusted	AD-MFI	AD-BG Adjusted	DR/DRSx	DQA	DQB	DPA	DPB	DR Serology	DQ Serology	DP Serology	RAD	Epitopes
Negative	398	1168	535		DQA1*01:01	DQB1*05:01				DQ5(1)		0.7440	
Negative	486	1010	523		DQA1*01:02	DQB1*06:04				DQ6(1)		0.9300	
Negative	459	1038	567		DQA1*01:02	DQB1*05:01				DQ5(1)		0.8080	
Negative	191	1029	307		DQA1*01:02	DQB1*05:02				DQ5(1)		0.6220	
Negative	274	655	352		DQA1*01:02	DQB1*06:02				DQ6(1)		0.7790	
Negative	696	1109	778		DQA1*01:03	DQB1*06:01				DQ6(1)		0.8940	
Negative	209	1201	285		DQA1*01:03	DQB1*06:03				DQ6(1)		0.5490	
Negative	322	1307	617		DQA1*01:04	DQB1*05:03				DQ5(1)		0.5320	
Negative	404	820	528		DQA1*01:04	DQB1*06:01				DQ6(1)		0.7650	
Positive	23933	27815	27446		DQA1*02:01	DQB1*03:02				DQ6(3)		0.8720	175E
Positive	23505	27413	27173		DQA1*02:01	DQB1*04:01				DQ4		0.8650	175E
Positive	20467	23894	23471		DQA1*02:01	DQB1*02:01				DQ2		0.8720	175E
Positive	19776	25311	24906		DQA1*02:01	DQB1*02:02				DQ2		0.7940	175E
Positive	14846	19869	19407		DQA1*02:01	DQB1*06:01				DQ6(1)		0.7650	175E
Positive	32552	35874	35576		DQA1*03:01	DQB1*03:01				DQ7(3)		0.9150	175E
Positive	29884	36140	35704		DQA1*03:01	DQB1*03:02				DQ8(3)		0.8370	175E
Positive	27106	38891	38285		DQA1*03:01	DQB1*04:02				DQ4		0.7080	175E
Positive	30016	36997	36515		DQA1*03:02	DQB1*03:01				DQ7(3)		0.8220	175E
Positive	29014	37006	36541		DQA1*03:02	DQB1*02:02				DQ2		0.7940	175E
Positive	27510	37188	36631		DQA1*03:02	DQB1*03:02				DQ8(3)		0.7510	175E
Positive	27317	35105	34710		DQA1*03:02	DQB1*03:02				DQ9(3)		0.7870	175E
Positive	11142	14394	13790		DQA1*04:01	DQB1*04:02				DQ4		0.8080	175E
Positive	10419	14132	13619		DQA1*04:01	DQB1*03:03				DQ9(3)		0.7650	175E
Positive	7650	11006	10596		DQA1*04:01	DQB1*04:01				DQ4		0.7220	175E
Negative	651	1332	777		DQA1*05:01	DQB1*03:01				DQ7(3)		0.8370	
Negative	226	670	316		DQA1*05:01	DQB1*04:01				DQ4		0.7150	
Negative	104	575	130		DQA1*05:01	DQB1*02:02				DQ2		0.7940	
Negative	12	513	15		DQA1*05:01	DQB1*02:01				DQ2		0.7790	
Positive	8919	11493	11038		DQA1*06:01	DQB1*03:01				DQ7(3)		0.8080	175E
Positive	8931	12461	12135		DQA1*06:01	DQB1*03:03				DQ9(3)		0.7360	175E
Positive	8190	11518	11127		DQA1*06:01	DQB1*04:02				DQ4		0.7360	175E

## RESULTS

Figure 1.

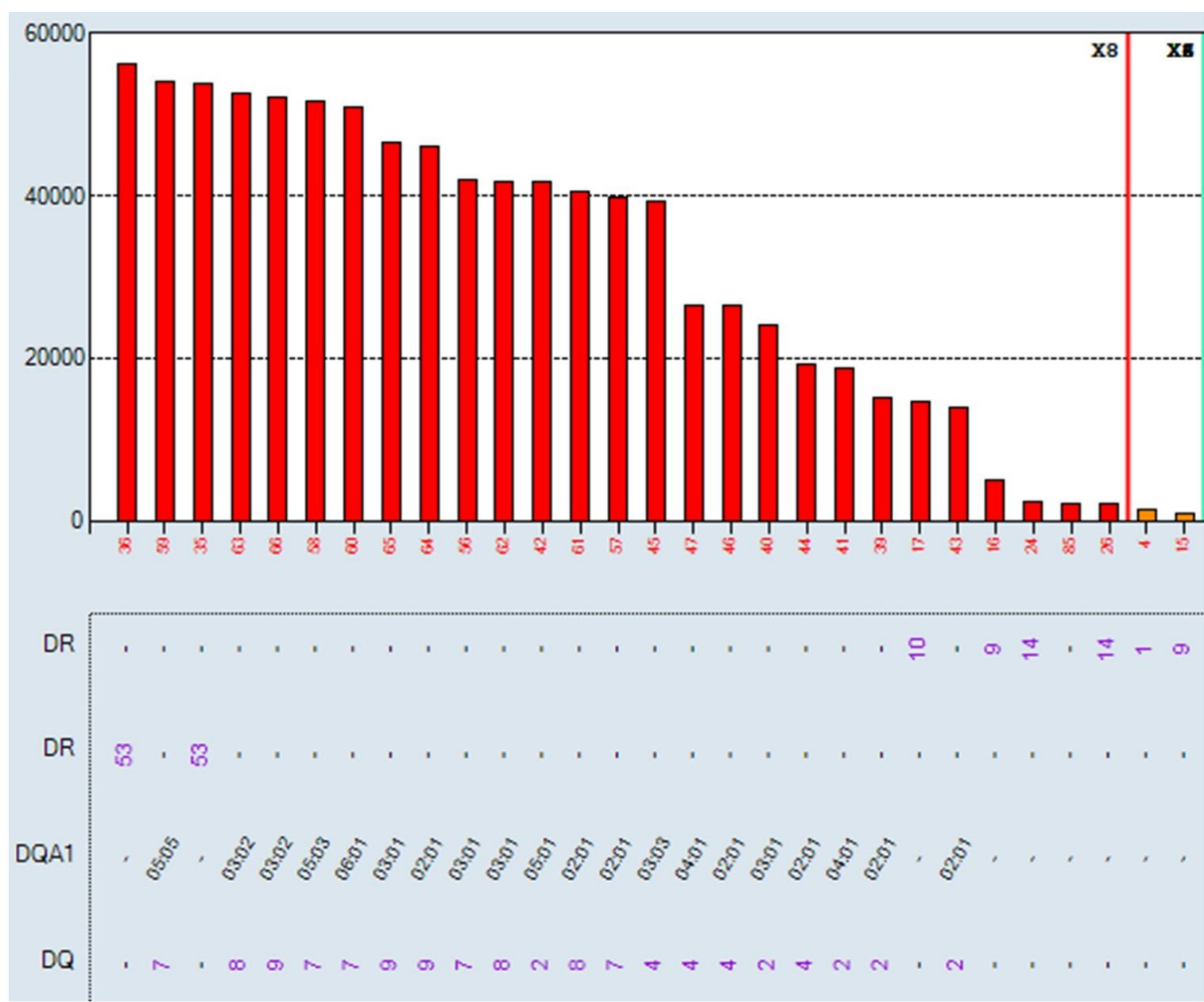


Figure 2.

Assignment	BG Adjusted	AD-MFI	AD-BG Adjusted	DR/DRSx	DQA	DQB	DPA	DPB	DR Serology	DQ Serology	DP Serology	RAD	Epitopes
Positive	3582	3689	36401		DQA1*01:01					DQ5(3)		0.9790	70R
Positive	3024	3162	31587		DQA1*03:02	DQB1*03:03				DQ9(3)		0.9620	91FT4
Positive	2977	3049	30258		DQA1*03:03	DQB1*03:03				DQ9(3)		0.9410	91FT4
Positive	2767	2945	29317		DQA1*03:02	DQB1*03:02				DQ9(3)		0.9410	91FT4
Positive	25702	29108	29108		DQA1*03:02	DQB1*03:01				DQ7(3)		0.8830	91FT4
Positive	24930	27932	27924		DQA1*03:03	DQB1*03:03				DQ7(3)		0.8830	91FT4
Positive	24705	29411	29307		DQA1*03:03	DQB1*03:03				DQ7(3)		0.8490	91FT4
Positive	24170	29479	29215		DQA1*03:03	DQB1*03:02				DQ9(3)		0.9220	91FT4
Positive	22454	26978	26867		DQA1*04:01	DQB1*03:03				DQ9(3)		0.8350	91FT4
Positive	21857	27514	27424		DQA1*06:01	DQB1*03:03				DQ9(3)		0.7970	91FT4
Positive	19508	25947	25941		DQA1*04:03	DQB1*03:03				DQ4		0.7920	91FT4
Positive	17957	18599	18586		DQA1*03:03	DQB1*04:02				DQ4		0.9590	91FT4
Positive	11628	14813	14589		DQA1*04:01	DQB1*04:02				DQ4		0.7970	91FT4
Positive	11644	12995	12767		DQA1*02:01	DQB1*04:01				DQ4		0.9120	91FT4
Positive	11497	15100	14976		DQA1*04:03	DQB1*03:03				DQ4		0.7920	91FT4
Positive	1087	10831	10723		DQA1*03:03	DQB1*03:02				DQ2		0.9220	91FT4
Positive	9738	13742	13525		DQA1*05:01	DQB1*04:01				DQ4		0.7290	91FT4
Positive	9442	12494	12294		DQA1*06:01	DQB1*04:02				DQ4		0.7680	91FT4
Positive	7481	8147	7999		DQA1*03:03					DQ10		0.9440	70R
Positive	6937	7973	7856		DQA1*02:01	DQB1*03:01				DQ2		0.8830	91FT4
Positive	6514	7517	7453		DQA1*02:01	DQB1*03:01				DQ2		0.8740	91FT4
Positive	6038	7214	7146		DQA1*05:01	DQB1*03:01				DQ2		0.8450	91FT4
Positive	5910	7098	6993		DQA1*03:03	DQB1*03:02				DQ2		0.8490	91FT4
Positive	5795	4668	4595		DQA1*02:01	DQB1*06:01				DQ9(1)		0.8180	91FT4
Positive	2303	2468	2398		DQA1*03:01					DQ9		0.9980	70R
Positive	1596	1980	1911		DQA1*01:03					DQ11(5)		0.8390	70R
Positive	1085	1242	1118		DQA1*03:02					DQ15(5)		0.9790	70R
Positive	998	1159	1029		DQA1*04:01					DQ14(5)		0.9790	70R
Positive	937	1149	994		DQA1*04:04					DQ14(4)		0.9410	70R
Positive	865	1113	989		DQA1*05:01	DQB1*04:02				DQ11(1)		0.8740	
Negative	628	980	739		DQA1*01:02	DQB1*06:04				DQ9(1)		0.8540	

Table 1.

DQA1 allele	DQB1 allele	Frequency (%)*
DQA1*03:01	DQB1*05:01	0.78
DQA1*03:01	DQB1*05:03	0.26
DQA1*04:01	DQB1*05:01	0.26
DQA1*05:01	DQB1*05:01	0.26
DQA1*05:01	DQB1*05:02	0.26
DQA1*05:02	DQB1*05:03	0.26
DQA1*06:01	DQB1*05:03	0.26
DQA1*02:01	DQB1*06:02	0.26
DQA1*02:01	DQB1*06:03	0.26
DQA1*02:01	DQB1*06:05	0.26
DQA1*03:01	DQB1*06:01	0.52
DQA1*03:01	DQB1*06:02	0.52
DQA1*03:01	DQB1*06:03	0.26
DQA1*05:01	DQB1*06:01	0.26
DQA1*05:01	DQB1*06:02	0.26
DQA1*05:01	DQB1*06:03	0.26
DQA1*05:01	DQB1*06:09	0.26

\*Frequency: Total number of copies of the haplotype in the United States population sample (Haplotypes /2n) shown in percentages (%) per AlleleFrequencies.net

Case 2: A female patient with history of pregnancy and previous kidney transplant was being evaluated for her second transplant. She exhibited significant class II reactivity in OL-SAB (**Fig 3**).

However, while all the DQ4 beads were positive by OL-SAB, LC-SAB showed a single DQ4-DQA1\*05:01 bead that was completely negative (**Fig 4**).

This confirmed that her antibodies were directed to the 175E epitope of the DQA1\*02/03/04/06 chains and not to the DQ beta proteins. Two (0.12%) of 1,714 patients with LC-SAB testing have confirmed antibodies to the 175E epitope.

## REFERENCES

1. Gonzalez-Galarza FF, McCabe A, Santos EJ, Jones J, Takeshita LY, Ortega-Rivera ND, Del Cid-Pavon GM, Ramsbottom K, Ghattaoraya GS, Alfirevic A, Middleton D and Jones AR

Allele frequency net database (AFND) 2020 update: gold-standard data classification, open access genotype data and new query tools

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