HLA Detective Work: DQA1*05:01 False Positive ?

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INTRODUCTION

A patient #1 was screened for HLA antibodies (DSA - Donor Specific Antibody) as part of the workup for cardiac transplant. Testing resulted in detection of DQA1*05:01/DQB1*02:01 DSA at greater than 15,000 MFI, however, other DQB1*02 beads were negative based on transplant center criteria.

Listing DQA1*05:01 as an avoid in UNOS results in a CPRA = 41%.

Discussion with the transplant center and limitation of the potential donor pool caused our laboratory to further investigate and validate the presence of the DQA1*05:01 DSA.

MATERIALS & METHODS

Initial DSA testing was performed using One Lambda (OLI) Thermo Fisher, LABScreen™ Single Antigen Class I & II reagents.
Because the One Lambda LABScreen™ Single Antigen Class II panel contains only one bead that expresses the DQA1*05:01 allele.

DSA testing using a titer at 1:16 was performed using One Lambda Labscreen™ Single Antigen Class II panel.

C1q testing was performed using One Lambda C1qScreen Assay kit.

A review of the Werfen LIFECODES® Single Antigen Class II panel was done. This panel contained 4 DQA1*05:01 beads with different DQB1 alleles: DQB1*04:01, *03:01, *02:02, & *02:01.
Additional DSA testing was performed using Werfen LIFECODES® Single Antigen Class II ID panel.

Surrogate flow cytometry crossmatches were performed with donors that had HLA typing of DQA1*05:01/DQB1*02:01. Flow crossmatches were performed on a Becton Coulter CytoFlex Cytometer.

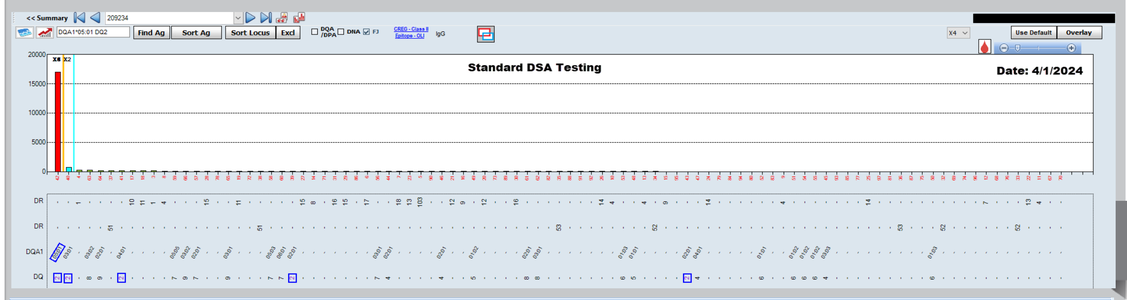
Figure 1. Patient #1 DSA Comparison & Crossmatch Results

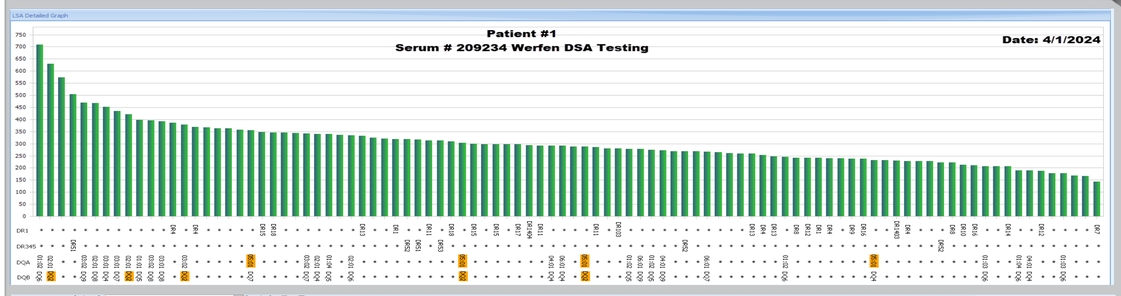
			One Lambda LabScreen™ (OLI)						Werfen LIFECODES®						
			OLI LabScreen™ SA		OLI LabScreen™ SA Titers		OLI LabScreen™ SA-C1q				FCXM				
			HLA Class II		HLA Class II - 1:16		HLA Class II		HLA Class II						
Patient	Serum #	Date	Specificity	MFI	Specificity	MFI	Specificity	MFI	Specificity	MFI	Date	T-cell	B-cell	Donor ID	Donor HLA DSA
#1	205599	1/8/24	DQA1*05:01/DQB1*02:01	25593			DQA1*05:01/DQB1*02:01	33808			4/1/24	Neg	Neg	1	DQA1*05:01/DQB1*02:01
	209211	3/29/24	DQA1*05:01/DQB1*02:01	19305	DQA1*05:01/DQB1*02:01	12264	DQA1*05:01/DQB1*02:01	14024			4/1/24	Neg	Neg	1	DQA1*05:01/DQB1*02:01
	209215	3/30/24	DQA1*05:01/DQB1*02:01	22825	DQA1*05:01/DQB1*02:01		DQA1*05:01/DQB1*02:01	11840	DQA1*05:01	440	4/4/24	Neg	Neg	2	DQA1*05:01/DQB1*02:01
											4/4/24	Neg	Neg	3	DQA1*05:01/DQB1*02:01
	209234	4/1/24	DQA1*05:01/DQB1*02:01	22865	DQA1*05:01/DQB1*02:01	9212	DQA1*05:01/DQB1*02:01	18767	DQA1*05:01	220	4/1/24	Neg	Neg	2	DQA1*05:01/DQB1*02:01
											4/4/24	Neg	Neg	3	DQA1*05:01/DQB1*02:01

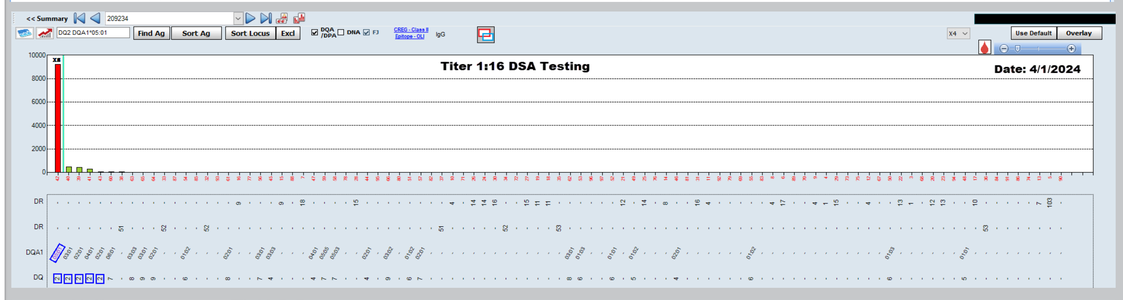
Figure 2. Additional Patients DSA Comparison

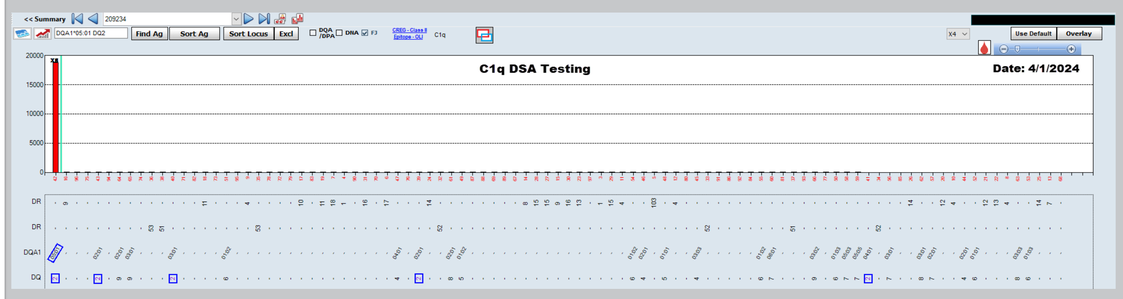
				One Lambda LabScreen™		Werfen LIFECODES®	
				HLA Class II		HLA Class II	
Patient	Pt. Category	Serum #	Date	Specificity	MFI	Specificity	MFI
#2	Renal	207239	2/12/2024	DQA1*05:01/DQB1*02:01	4918	DQA1*05:01	604
#3	Renal	175862	10/25/2024	DQA1*05:01/DQB1*02:01	17788		
		210278	4/19/2024	DQA1*05:01/DQB1*02:01	16100	DQA1*05:01	477
#4	Cardiac	210340	4/22/2024	DQA1*05:01/DQB1*02:01	15251	DQA1*05:01	437
		215949	Neg	DQA1*05:01/DQB1*02:01	12560	DQA1*05:01	189
#5	Cardiac Renal	210330	4/18/2024	DQA1*05:01/DQB1*02:01	14290	DQA1*05:01	830
#6	Cardiac Renal	152821	9/23/2019	DQA1*05:01/DQB1*02:01	11264	DQA1*05:01	551
		206536	1/30/2024	DQA1*05:01/DQB1*02:01	6951	DQA1*05:01	396
#7	Renal	208747	3/15/2024	DQA1*05:01/DQB1*02:01	3038	DQA1*05:01	220

Figure 3. Patient #1 Histograms









RESULTS

Various DSA detection methods were utilized to determine the biological activity of the DQA1*05:01 DSA.
DSA testing performed at 1:16 titer was **positive** at greater than 15,000 MFI.
C1q testing was also **positive** for the DQA1*05:01/DQB1*02:01 combination at greater than 15,000 MFI.
Screening with LIFECODES® reagents were **negative** for all DQA1*05:01/DQB1 combinations.
Finally, surrogate crossmatches with donors that expressed the DQA1*05:01/DQB1*02:01 were all B-cell negative (and T-cell negative).

An additional six patients that exhibited the DQA1*05:01 DSA (without corresponding DQB1*02 DSA) were tested using Werfen LIFECODES®. All six patients were negative.

CONCLUSIONS

Using all the data from HLA Class II single antigen testing and surrogate crossmatches, the decision was made to not list the DQA1*05:01 as an UNOS avoid. Listing the DQA1*05:01 would have resulted in approximately 40% of all donors being excluded from the pool of potential cardiac donors.
In addition, if standard testing indicates only the presence of DQA1*05:01 DSA, our Laboratory reflexes to testing on the Werfen LIFECODES® Class II Single Antigen panel.
This type of patient advocates the need for HLA laboratories to have multiple sources for HLA antibody screening. This patient also demonstrates the value of surrogate crossmatches. Fortunately, the patient was successfully transplanted (with donor that did not have DQA1*05:01/DQB1*02:01) and DSA monitoring has been negative.