Unmasking False-Positive anti-HLA reactivity in Luminex SAB assays using Adsorption/Elution and Surrogate Flow Cytometry Crossmatch

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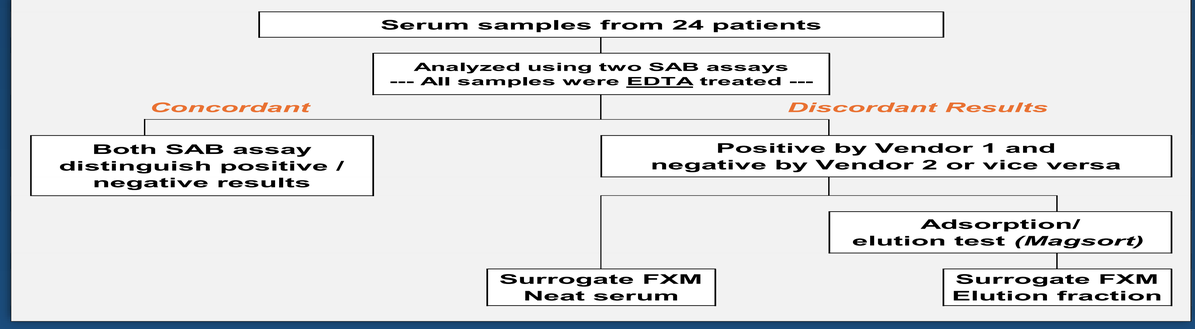
Introduction

Luminex Single Antigen Bead (SAB) assays are the standard method for the detection and monitoring of anti-HLA antibodies. However, HLA denaturation on the beads can expose cryptic epitopes, potentially leading to false-positive reactivity and contributing to the frequently observed discordant results between SAB assays from different vendors.

Objective

To investigate discordant results between SAB assays from different manufacturers and assess their impact on virtual crossmatch (VXM) interpretation and post transplant monitoring.

Methods



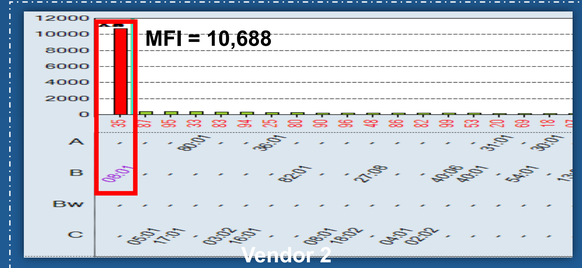
Results

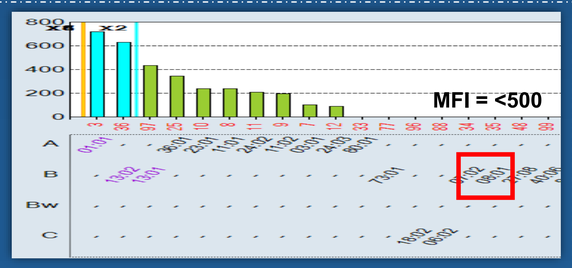
- Discordant HLA class I and II specificities are summarized in Table 1. Patient 1 showed true-positive results in both assays, whereas Patient 2 demonstrated a false-positive result with Vendor 1.
- Patient 5 exhibited a false-positive result with Vendor 2. Confirmation by adsorption/elution was not possible due to unavailable specificity. Surrogate FXM was performed using donors expressing the same specificity or homozygous allele.
- Patients 3, 4, 6, 7, and 8 showed low-level reactivity that may represent weak false-positive results in both assays. These findings could not be confirmed by adsorption/elution or surrogate FXM because of low MFI values.

Patient 2

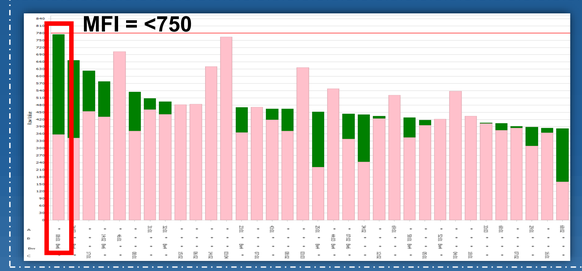
Neat Serum | **Elution Fraction**

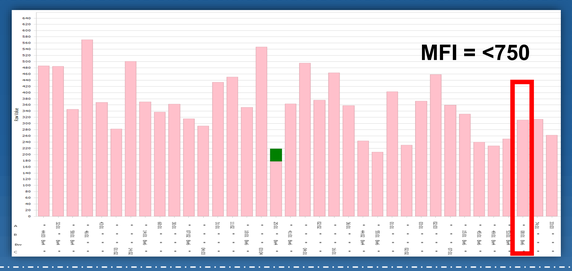
Vendor 1





Vendor 2

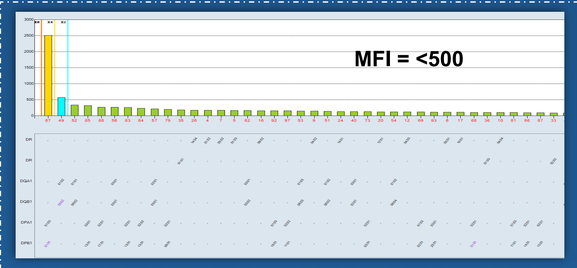




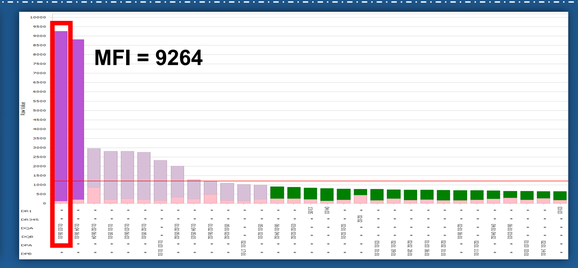
Patient 5

Neat Serum

Vendor 1



Vendor 2



Surrogate FXM (using three different donors)

			Neat Serum					
			Donor 1 DQB1*06:01 DQA*01:03 DQB1*06:02 DQA*01:02		Donor 2 DQB1*06:02 DQA*01:02 DQB1*06:04 DQA*01:02		Donor 3 DQB1*06:02 DQA*01:02 DQB1*05:03 DQA1*01:04	
Specificity	Vendor 1 MFI[a]	Vendor 2 MFI[a]	T Cell FXM	B Cell FXM	T Cell FXM	B Cell FXM	T Cell FXM	B Cell FXM
DQA1*01:03, DQB1*06:01	<500	9264	Negative	Negative	Negative	Negative	Negative	Negative

Table 1. Discordant HLA specificities identified between the two single-antigen bead (SAB) platforms

[a] SAB Test MFI Positive cutoff: Vendor 1 > 500; Vendor 2 > 750

		Neat Serum				Elution Fraction			
Patient	Specificity	Vendor 1 MFI [a]	Vendor 2 MFI [a]	T Cell FXM	B Cell FXM	Vendor 1 MFI [a]	Vendor 2 MFI [a]	T Cell FXM	B Cell FXM
1	B*08:01	8680	8296	Positive	Positive	5240	3646	Positive	Negative
2	B*08:01	10688	<750	Negative	Negative	<500	<750	Negative	Negative
3	B*08:01	2142	<750	Negative	Negative	<500	<750	Negative	Negative
4	B*44:02	2244	<750	Negative	Negative	<500	<750	Negative	Negative
5	DQA1*01:03, DQB1*06:01	<500	9264	Negative	Negative	No Data	No Data		
6	DQA1*01:01, DQB1*05:01	<500	2820	Negative	Negative	<500	<750	Negative	Negative
7	DQA1*01:03, DQB1*06:03	<500	2813	Negative	Negative	<500	<750	Negative	Negative
8	DQA1*02:01, DQB1*02:02	<500	1932	Negative	Negative	<500	<750	Negative	Negative

Conclusions

1. Surrogate flow cytometry crossmatches confirmed false-positive anti-HLA class I and II antibody reactivities in both SAB platforms, highlighting that each assay has inherent limitations.
2. These findings underscore the need to improve SAB assay to ensure accurate VXM interpretation and post-transplant monitoring of donor-specific antibodies.
3. The use of adsorption/elution techniques and surrogate FXM may improve the SAB assay performance and immunological risk assessment.

References

1. Matias EG, Austin DE, Franca KC, Maguire C, Lockhart JS, Lantz CW, et al. Not All Mismatches Were Created Equal: Empirical Identification of Regions Within HLA-DQ Molecules That Harbor the Highest Immunogenicity. Transplantation. 2026.
2. Piazza A, Rombola G, Maggiore U, Ciappi D, Cozzi E, de Stefano MC, et al. A comprehensive comparative assessment of mean fluorescence intensity of luminex single antigen bead tests between laboratories and commercial platforms: A report from the Italian Histocompatibility Network. Hum Immunol. 2025;86(6):111611.
3. Grenzi PC, de Marco R, Silva RZ, Campos EF, Gerbase-DeLima M. Antibodies against denatured HLA class II molecules detected in luminex-single antigen assay. Hum Immunol. 2013;74(10):1300-3.
4. Vu TTM, Wang R, Mathew S, Abeywardana T, Beltran-Lemus M, Ma V, et al. Using MagSort to identify eplets in a high PRA patient serum and revealing a novel HLA-A*01:01 specific binding pattern. Hum Immunol. 2025;86(5):111344.

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