The HLA A2 DSA Mystery: The Missing Link between Single Antigen Bead (SAB) and Cell-based Capture-P Platelet Crossmatch Assays

Abdelhamid Liacini[1], Marina Ohashi[1], Paul Sibilia[1], Eric Lee[1], Noureddine Berka[1], Jennifer Brissette[2], Neng Yu[12]

[1] Histocompatibility Laboratory Services, American National Red Cross, Dedham, MA

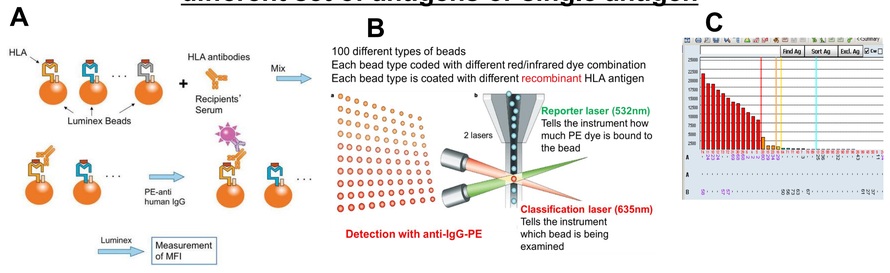
[2] UMass Memorial Medical Center, Worcester, MA

BACKGROUND

- Platelet refractoriness is a condition where patients fail to respond to platelet transfusions despite receiving an adequate number of platelets.
- It leads to persistent thrombocytopenia and increases the risk of bleeding complications.
- Common in patients with hematological diseases (e.g., leukemia, bone marrow disorders) and those undergoing chemotherapy who need repeated transfusions.
- Most cases are due to non-immune factors, but about 20% are immune-mediated, often from alloimmunization against HLA antigens.
- Approaches include selecting compatible platelet units using antibody specificity prediction, which avoids donor antigens targeted by the patient's antibodies.
- Early 2000s improvements in HLA antibody detection allowed precise identification of antibodies against individual HLA antigens (**(Figures 1&2).**
- Donor-specific HLA antibodies (DSAs) are major drivers of immune-mediated refractoriness and are linked to graft rejection, delayed graft function, and positive crossmatches in transplant settings. While strong DSAs have been associated with reactivity in various crossmatch assays, unexpected findings can occur**.**

METHODOLOGY

Luminex: Multi-array, 3 color immunofluorescence Dual-colored beads each made with different ratio of two colors and each with a different set of antigens or single antigen



The figures 1A, B, C represent the principles underlying the Luminex bead assay. Each bead has one or more different types of human leukocyte antigen (HLA) molecules attached depending on the level of testing being performed. If the test serum contains an HLA antibody it will bind to the appropriate HLA molecule. The Luminex fluorocytometer which utilizes two lasers, one of which excites the fluorochrome in the bead and the other laser excites the Phycoerythrin (PE) bound to the detection antibody. The first readout therefore identifies the unique signal of the bead and hence the specificity of the bound HLA molecule, while the second readout indicates whether or not antibody is bound to the specific HLA molecule. The degree of fluorescence is expressed as a mean fluorescence intensity (MFI), which is normalized by taking into account the degree of fluorescence observed with an antibody negative serum and with beads to which no HLA molecule is attached.

A Capture-P platelet crossmatch

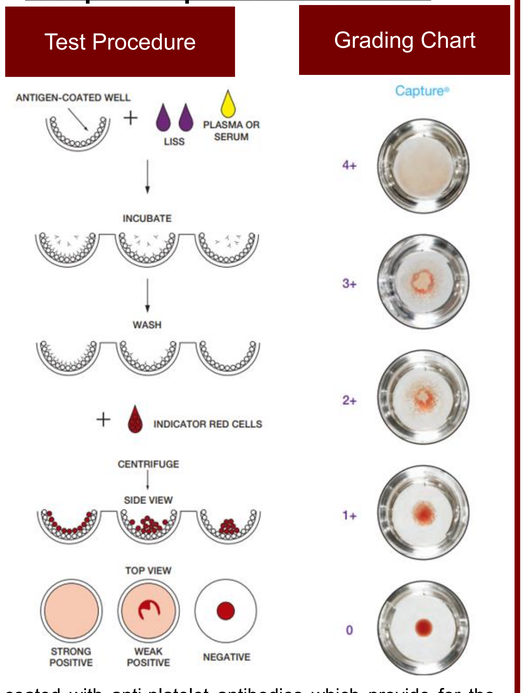


Figure 2: Test wells are coated with anti-platelet antibodies which provide for the specific binding of platelets when the source of platelets is platelet-rich plasma. The assay will detect platelet specific and HLA antibodies.

RESULTS

In this report, we describe a case of a 35-year-old female patient genotyped as HLA-A29, 34; B50, 53 with a history of transfusions was found to have strong HLA class I antibodies, including anti-A2 at an assay saturation level of 22,000 MFI (Table 1).

SAB Bead Number	HLA-A2 specificities	Normalized MFI	Raw MFI
004	A*02:01	22358	22504
006	A*02:06	21357	21502
005	A*02:03	21013	21126

Table1: Donor specific antibody to HLA A2 on a Luminex SAB assay expressed as mean fluorescence intensity (MFI):

However, despite HLA-A2 being a common antigen in the panel, multiple Capture-P platelet crossmatches (PXMs) were completely Negative **(Figures 2&3)**. This discordance between DSA presence and crossmatch results prompted further investigation. A surrogate flow cytometry crossmatch (FCXM) was subsequently performed and showed a strong positive reaction against an HLA-A2 donor. A 1:10 dilution study showed anti-A2 reactivity at 8,000 MFI and Negative PXMs. A 1:10 dilution study showed anti-A2 reactivity at 8,000 MFI and Negative PXMs.

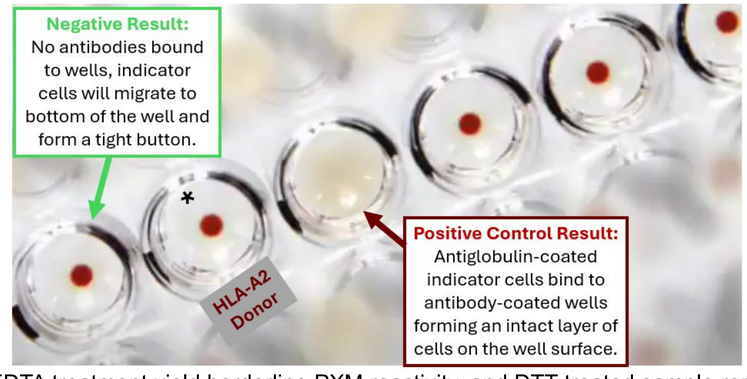


Figure 3: A Capture-P platelet crossmatch detecting antibody-mediated reactivity between patient serum and donor platelets. Unexpected findings were found where a patient with a strong **A2/ 22,000 MFI (1:10/ 8.000 MFI) DSA** did not show reactivity in the platelet crossmatch (Asterisk).

EDTA treatment yield borderline PXM reactivity, and DTT treated sample remained PXM Negative. Undoubtedly, the lack of reactivity in the PXM was due to interference(s) present in the patient's serum, preventing the binding of anti-human IgG coated indicator red blood cells to the immobilized platelets bonded with anti-A2 IgG. We were unable to pinpoint the cause. As a result, HLA-A, -B matched or antigen-negative platelets were suggested for transfusion and two non-A2 products were sent to the hospital. The HLA typing of two platelet products was **A31, A68; B44, B71 and A3, A11; B27, B47.**

CONCLUSIONS

This case highlights the importance of utilizing multiple testing platforms. Since PXM alone can yield false-negative results, and SAB alone may not fully reflect HLA antibody MFI tolerance, a combined approach using both PXM and SAB provides a more comprehensive strategy for managing highly sensitized refractory patients.