Selective bead group missing in HLA single antigen bead assay-Technical error or Assay limitation?

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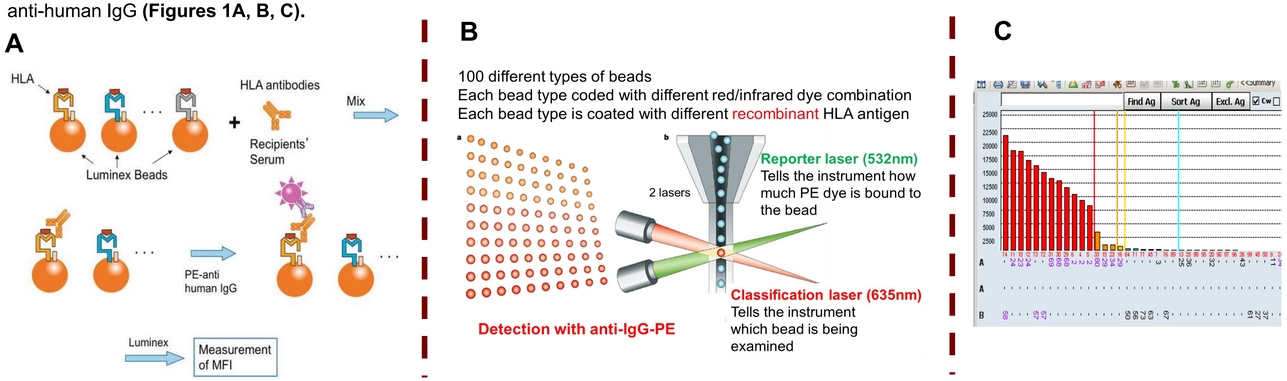
BACKGROUND

Advances in HLA antibody detection and specificity identification have translated into improvements in organ and Stem cell transplantation. Solid phase assays, e.g., single antigen bead (SAB) ((**Fig. 1A, B & C)**, have led to more precise patient immune profiling for donor selection. However, solid phase assays have commonly observed limitations which can make interpretation of results challenging. Specifically, the SAB assay is highly sensitive but has several limitations, including false positives from antibodies targeting denatured or non-native epitopes, false negatives due to limited allele coverage or the prozone effect, variability in antigen density, lot-to-lot differences, nonspecific background, and lack of functional assessment such as complement binding or antibody pathogenicity.

Here, we present an observation to add to the assay limitation watch list. We performed the HLA antibody analysis using the SAB method on a 62-year-old female stem cell transplant patient. A low C bead (<25) count was observed for all HLA-C beads while HLA-A and -B bead counts were normal. No other patients on the same run had low C bead counts. Repeated testing on samples collected from the patient's various time points yielded the same observation. The low HLA-C bead counts varied from 0-25, resulting in apparent false positive reactivity calls**.** Further analysis revealed that the patient's serum when treated with dithiothreitol (DTT), showed normal bead counts and no detectable anti-C reactivities **(Fig. 2, 3 & 4)**. It is hypothesized that anti-C IgM antibodies were likely responsible for the diminished C bead count. These antibodies bind to HLA-C antigen-coated beads, forming a substantial complex that may shield the bead and lead to its loss during the washing process. This case underscores the importance of considering IgM-mediated interference and using DTT as the mitigation agent. Most HLA labs are using EDTA to reduce SAB test interferences; recognizing EDTA treatment is not effective for IgM-associated interferences is crucial for accurate interpretation of HLA antibody analysis using the SAB method.

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 introduction of fluorescently labeled beads revolutionized HLA antibody testing during the 1990s. Commercial kits are available **(One Lambda, ThermoFisher)** which consist of beads impregnated with differing ratios of two fluorochromes resulting in a unique signal for each bead and which have one or several types of HLA molecules attached. The assay involves first the incubation of a patient's serum with the beads. If the patient has HLA antibodies the serum will react with the bead expressing the appropriate HLA molecule. After washing, the beads are incubated with a secondary antibody, usually with a phycoerythrin (PE)-labeled anti-human IgG **(Figures 1A, B, C).**



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 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.

RESULTS

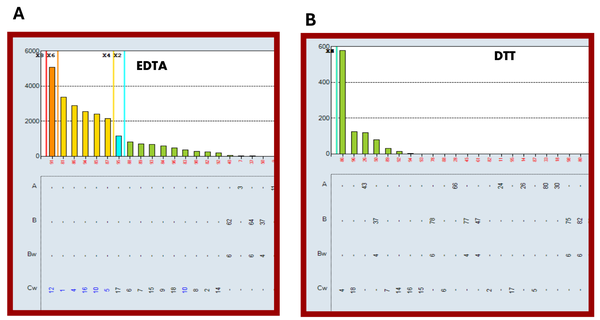
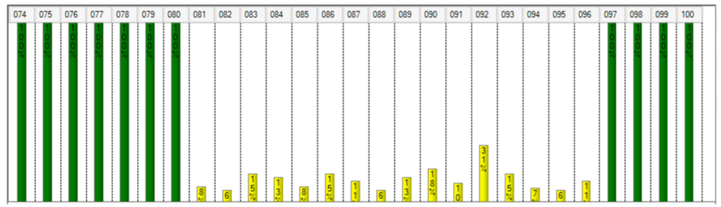


Figure 2: HLA class I SAB testing. A) The patient exhibited a recurrent pattern of no to low HLA-C beads resulting in false-positive results. B) The patient serum was DTT treated, and HLA-C bead counts recovered and became Negative.

A: EDTA treated serum



B: DTT treated serum.

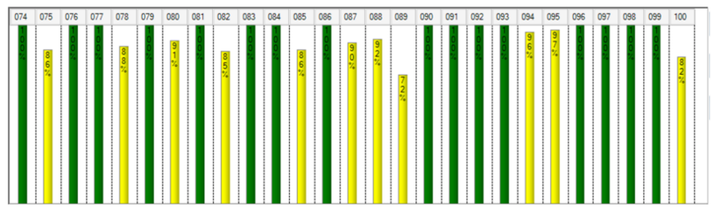


Figure 3: RUN CSV File Pattern, HLA-C antigen bead 081-096

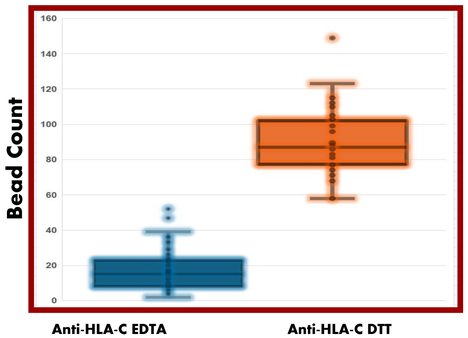


Figure 4: Pattern was characterized by low bead counts, particularly for HLA-C specificities. When the patient serum was DTT treated, we observed a significant increase in bead count.

CONCLUSIONS

This case demonstrate the importance of critical analysis and confirmatory testing in HLA antibody detection. Low bead counts can cause false positive calls. DTT treatment remains a vital tool in removing IgM related interference and should be used in addition to EDTA treatment.