



# The use of EDTA in the Luminex HLA antibody test assay improved treatment of an ECMO patient

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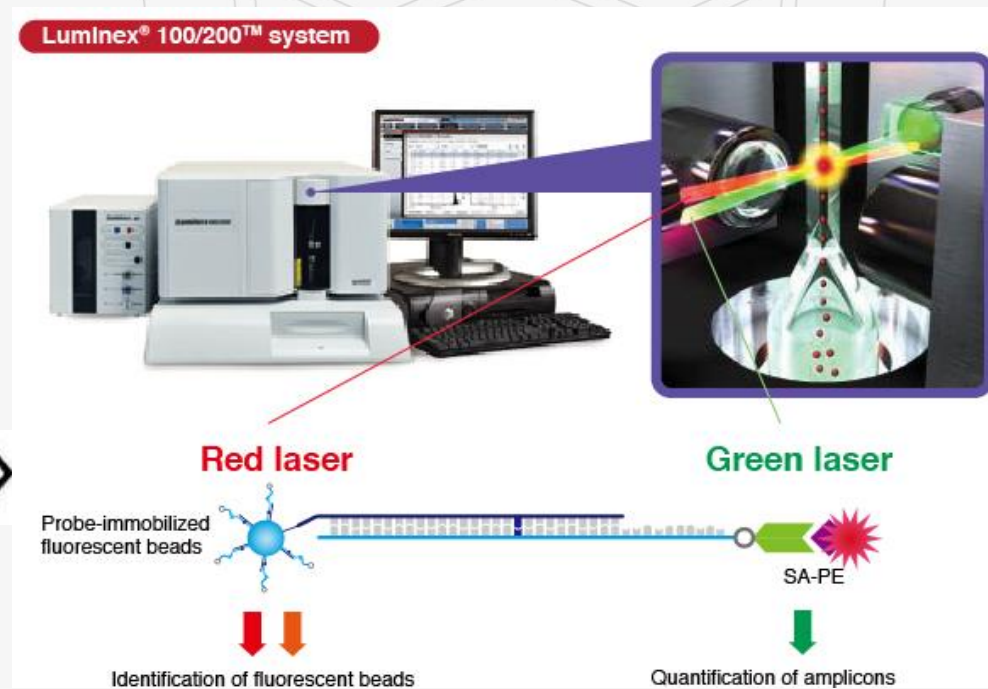
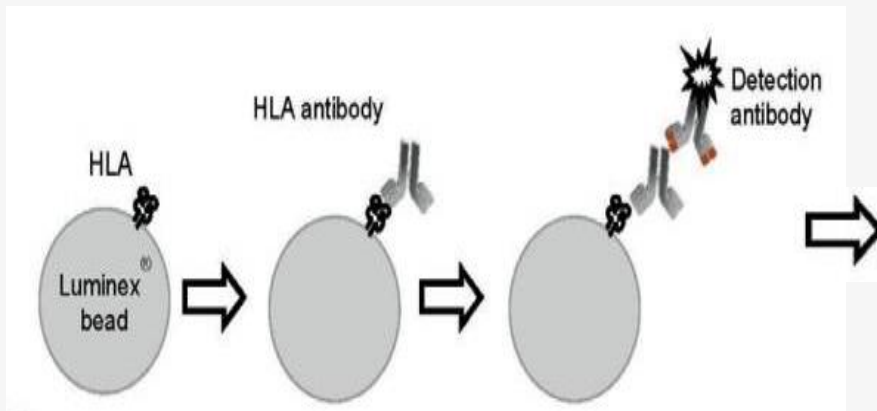
# Introduction – provision of platelets

- Patients require platelets;
  - Therapeutic - active bleeding
  - Prophylactic (preventative) - patients receiving treatments e.g chemotherapy, ECMO and surgery.
- **Extracorporeal membrane oxygenation (ECMO)**
  - ECMO is a cardiac support to a person whose heart is unable to provide an adequate amount of gas exchange to sustain life.
  - ECMO patients have difficulty maintaining platelets counts require platelet support.
- Platelet refractoriness - **Immunological**  
Preformed antibodies in recipient serum to antigens on platelets:
  - HLA (Human Leucocytes Antigens) class I specific antibodies
  - HPA (Human Platelet Antigens)



# HLA antibody Testing

- Samples sent to NHSBT Colindale for Platelet refractoriness
- HLA class I antibodies screening was performed by Luminex® technology using HLA class I single antigen beads.
- LUMINEX





# Case study: Platelet refractoriness due to HLA antibodies

- Patient: 41yr old male, receiving ECMO for cardiac failure
- Referred to H&I Colindale:
  - Poor response to ABO compatible pooled platelets
- HLA type : HLA-A\*03, A\*24, B\*07, B\*39
- HLA antibodies detected:
  - All HLA-A locus antigens **except A2, A68 and A69 and self antigens**
  - HLA-B locus antigens specificities (B8,18,27,38,40,42,44,48,54,55,56,64,65,73).
  - HPA was **negative**
- Due to the patient's antibody profile:
  - predicted not to increment with over 50% of pooled platelets
  - **Pre** transfusion count on random platelets :  $24 \times 10^9/L$
  - **Post** transfusion count on random platelets:  $26 \times 10^9/L$



## Case: Increments following HLA selected platelets

- Patient Supported with HLA selected platelets
- Received a number of match grade platelets.

**Patient HLA type: A\*03 A\*24 B\*07, B\*39**

ABO Rh	HLA TYPE	MATCH GRADE	Pre tfn Counts 10 <sup>9</sup> /L	Post tfn Count 10 <sup>9</sup> /L
A Negative	A*03 A*24 B*07 B*15 (62)	B1	-	-
O Positive	A*03, A*24, B*07, B*07	A	27	56
AB Positive	A*03, A*32, B*07, B*51	B2	66	59
O Positive	A*03, A*03, B*07, B*07	A	-	-
A Positive	A*03, A*03, B*07, B*15 (62)	B1	52	56
O Positive	A*03, A*03, B*07, B*07	A	45	57
O Positive	A*03, A*03, B*07, B*35	B1	55	59
O Positive	A*02, A*03, B*07, B*15 (62)	B2	57	42
A Positive	A*03, A*03, B*07, B*07	A	41	53

- Good increments achieved with all match grades platelets.
- New samples sent for HLA antibody monitoring.



# HLA antibody Testing – EDTA protocol

- Following reports on use of EDTA

*Schnaidt M, Weinstock C, Jurisic M, et al. HLA antibody specification using single antigen beads - a technical solution for the prozone effect. Transplantation 2011; 92: 510-515.*

- Reports suggest that addition of EDTA in serum can decrease complement mediated prozone effect.
- The reasons of prozone effect is unknown but complement components have been suggested to be causing interference in binding of antibodies.

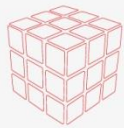
- Samples sent to NHSBT Colindale for HLA class I antibody monitoring was tested using the EDTA protocol (in wash buffer).



# EDTA protocol results

- HLA antibodies detected:
  - All HLA-A locus antigens **except patients own HLA type**
  - All HLA-B locus antigens **except patients own HLA type.**
- Due to patients new antibody profile in the presence of EDTA:
  - Platelet donor pool decreased
    - HLA-A\*02 is present in over 40% of platelet donors.
    - HLA-B\*44 is present in over 30% of platelet donors
- HLA type of the patient has a major bearing on the availability of HLA matched platelets.





# Conclusion

- Antibody testing with the addition of EDTA revealed that the patient actually had a more extensive range of HLA antibodies.
- This case highlighted;
  - Luminex® -based single antigen HLA antibody screening can be affected by prozone inhibition.
  - The addition of EDTA to serum of highly immunized patients mitigates the complement-mediated prozone effect.
  - Improves detection of HLA antibodies and enables more effective patient treatment.
- All refractory patients at NHSBT are now routinely tested for HLA antibodies using the EDTA protocol.