VALIDATION OF A BEAD-BASED ANTI-ABO-A ASSAY BETWEEN TWO CENTRES:
One of these things is a lot like the other

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Conclusions

- Despite the use of different red blood cell (RBC) titre methods at each centre, the proposed safe MFI threshold for ABO-A2-incompatible (ABO-A2i) transplant was similar for both centres
- Levels of clinically relevant anti-A-II antibody were similar in many patients deemed *ineligible* for ABO-A2i donors compared to the *eligible* group
- Presence of anti-A-III and A-IV antibodies and other irrelevant RBC antibodies may be responsible for non-donor specific titre reactivity
- Bead-based ABO antibody assay overcomes RBC titre limitations improving access to ABO-A2 organs

Introduction and Background:

Blood group ABO-B and ABO-O renal transplant candidates are safely transplanted with non-A1 (ABO-A2*) blood group kidneys (Bisen et al, AJT, 2024). This ABO-A2-incompatible (ABO-A2i) transplant practice increases access for transplant to ABO-B and O blood group patients who have longer wait times than ABO-A and ABO-AB candidates. ***Difficulty establishing anti-A titre thresholds and/or determining patient eligibility have been cited as barriers to implementing these ABOi programs.***

To determine eligibility for ABO-A2 organs, the level of anti-ABO-A antibody must be measured. Red blood cell (RBC) titres are currently used but there are several issues with this methodology:

- RBC titres are known to be plagued by lack of both reproducibility and method standardization.
- ABO-A1 RBC are used for agglutination titres but these cells have A-II, III, and IV glycans whereas ABO-A2 kidneys are decorated with only A-II glycans

We developed and published a Luminex, single antigen, subtype-specific bead-based assay and found that many eligible patients are denied access to ABO-A2 allografts; these data are included here as Centre 1

AIM: To test the potential antibody threshold for anti-A-II antibodies observed in two different centres

** The term ABO-A2 will be used to describe lectin typed non-A1 phenotype here as the reagent red cells use this terminology although this term is not accurate unless the individual has been genotyped.*

Methods:

- Eligibility for ABO-A2i transplant was determined in each centre by their RBC titre method (**Table 1**)
- Anti-A subtype specific antibodies were measured using a Luminex assay as previously described
- Levels of IgG anti-A-II antibodies were grouped by eligibility criteria, as determined by titre
- The reactivity to each subtype coupled bead was measured by mean fluorescence intensity (MFI)
- Data were analysed using GraphPad Prism.

Luminex antibody testing details can be found in this link:

Results:

Centre 1 had more ABO-O candidates that Centre 2(**Table 1**)

In the Centre 1 cohort, as previously reported, the highest anti-A-II IgG MFI in the eligible group was 32000 and 55% of the ineligible group had MFI values less than 32000 MFI

In Centre 2, the highest MFI in the eligible group was 25700 and 67% of the ineligible candidates had anti-A-II IgG antibodies less than 25700

Centre 2 patients had lower anti-A-II levels than Centre 1 patients in both eligible ($p<0.02$) and ineligible groups ($p<0.001$), however there was much overlap between both groups (**Figure 1**)

Table 1: There were differences in RBC titre method and patient populations studied at each centre. Centre 1 had more ABO-O candidates assessed for compatibility with ABO-A2i living donors than Centre 2. Both centres assessed ABO-B candidates for ABO-A2i transplant as per Kidney Allocation System (KAS) policy for deceased donors.

Centre	Titre Method	Titre Threshold	Patients Deemed Eligible	Patients Deemed Ineligible
Centre 1	DTT, RT, gel card	≤1/4	n=66 (39% ABO-O) (61% ABO-B)*	n=75 (62% ABO-O) (38% ABO-B)*
Centre 2	DTT, 37°C, AHG, gel card	≤1/8	n=77 (0% ABO-O) (100% ABO-B)*	n=51 (16% ABO-O) (84% ABO-B)*

*ABO-B individuals have lower levels for IgG anti-A than ABO-B individuals thus creating potential differences in levels of IgG antibodies between Centre 1 and 2

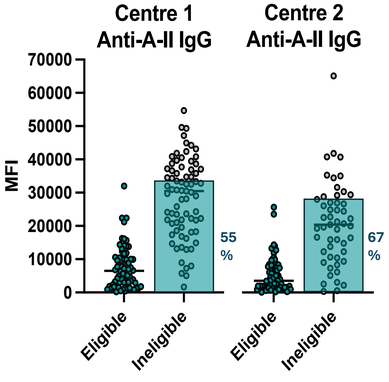

Figure 1: MFI levels of IgG anti-A-II antibodies in eligible and ineligible candidates at Centre 1 and Centre 2; each centre used different agglutination titre methods and titre thresholds to determine ABO-A2i eligibility. Many (55%) of ineligible candidates at Centre 1 had anti-A-II MFI in a comparable range to eligible candidates. Similarly, 67% of candidates deemed ineligible by titre testing at Centre 2 had anti-A-II levels that overlapped with eligible patients.

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