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## O010 A novel microfluidic immunoassay for in-solution quantification of alloantibody affinity and concentration in transplantation and beyond

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**Introduction:** Antibody characterisation is fundamental in transplantation and infectious diseases, but current immunoassays cannot determine two fundamental antibody properties, affinity ($K_D$) and concentration ([Ab]). We aimed to overcome these limitations to allow in-depth profiling of antibodies directly in sera and provide insights into clinical translation.

**Methods:** Using a microfluidic diffusional sizing-based strategy, we developed microfluidic antibody affinity profiling (MAAP), a novel in-solution immunoassay that simultaneously determines $K_D$ and [Ab] directly in serum. MAAP was developed and validated using the HLA-alloantibody system and applied in HLA Ab-incompatible (HLAi) transplantation and in anti-SARS-CoV-2 immunity.

**Results:** MAAP enabled quantification ($K_D$ and [Ab]) of alloantibody-HLA interactions in both purified and alloantibody-spiked sera. We demonstrated that transplant single-HLA-bead (SAB) immunoassays were avidity and [Ab]-dependent, cellular immunoassays (flow-cytometry and complement-dependent-cytotoxicity) were $K_D$ and [Ab]-dependent, antibody-mediated cytotoxicity was proportional to antibody-HLA $K_D$, and micromolar antibody-HLA interactions were functionally insignificant despite high SAB signal. In HLAi transplants, MAAP differentiated clinically significant donor-specific-alloantibodies (leading to rejection) from those tolerated despite similar SAB assay output and provided insights into memory re-activation and immune-monitoring post-transplantation. In SARS-CoV-2, MAAP showed wide variation in anti-RBD antibody $K_D$ in convalescent sera (n=34), good correlation with serum neutralisation capacity ($p<0.001$), and evidence of affinity maturation 3-months post-infection (despite [Ab] reduction). In vaccinated sera (n=17), anti-RBD antibody $K_D$ was significantly weaker against Omicron than wild-type ($p<0.001$), providing insights into variant immune-escape strategies.

**Conclusion:** This work outlines a path towards in-depth antibody profiling and demonstrates the importance of antibody abundance and affinity in clinically relevant humoral immunity.