**Generation of a validated suspension bead array assay for exploratory and high-throughput profiling of human plasma**

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**Introduction and aim**

The composition of the human plasma proteome is unique and yet complex, hence no single technique is adequate for its complete analysis. Antibody-based bead array assays facilitate fast, flexible and multiplexed screening of hundreds of proteins in plasma for the discovery of novel protein signatures. Affinity reagents including antibodies have been widely exploited for exploratory approaches however reagent performance is dependent on the context of the scientific application and many discovery projects fail to deliver data for every protein studied. Here we established a validated multiplexed suspension bead array (SBA) for the discovery of novel protein signatures in bodily fluids utilizing the resource of antibodies from the Human Protein Atlas (HPA).

**Methods**

Multiple SBA’s were generated using xMAP beads covalently coupled to up to >20,000 HPA antibodies. Over 4,000 human plasma samples from various disease cohorts (including cancer, cardiovascular, neurological disease) were directly labelled with biotin to facilitate a systematic and high-throughput screening approach, applied to the SBA after heat treatment. Measurements were performed using a FlexMAP 3D instrument and antibodies with MFI values 2x SD + mean above bare beads were initially classified as reacting with proteins in plasma. Confidence of antibody reactivities were subsequently assessed by correlation of antibody pairs to same target protein, correlation with GWAS data, target confirmation by immuno-capture mass-spectrometry and use of antibody development of sandwich immunoassays.

**Results**

The performance of antibodies in a given context is crucial for obtaining high-quality reliable data. From >20,000 antibodies investigated 384 antibodies targeting 200 proteins were selected for our validated panel. Gene ontology analysis of antibodies included in our array show enrichment in pathways involved in cellular, metabolic, developmental, immune process as well as biological regulation, localization, adhesion, reproduction and cell killing pathways. To ensure confidence in our data we confirmed the stability of measuring proteins across repeated assays. We also included pairs of antibodies, against 173 protein targets and confirmed pairs showed a good correlation. Protein levels were assessed according to sample collection date. The majority of proteins had stable protein levels across seasons, indicating the plasma proteome within a given sample is relatively stable across the seasons.

**Conclusion**

In summary we have established a validated assay for the exploratory profiling of up to 200 proteins in a stratified pipeline. Our exploratory approach in a healthy cohort revealed the majority of proteins were stable over a one year period. Our intention is to use and expand this large single coupling as a validated pipeline as a service-package offered to collaborators to screen their disease cohorts. The identical screening configuration illustrates an opportunity to compare multiple independent cohorts on an identical configuration for novel biomarker discovery with increased precision and decreasing the technicalities associated with generating novel highly-multiplexed SBAs.