**Affinity proteomics on beads**

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**Background**

Affinity reagents serve as versatile tools to build immunoassays to increase our understanding of human biology. With analytical as well as technological advances emerging, the performance of affinity reagents in a given assay and sample context remains key to the generation of high-quality data. There are several examples of how current affinity efforts have profiled human samples in different disease areas, but strategies need to go beyond the current set of markers, hence discover and validate novel targets and assays. In this way, multiplexed immunoassays will remain useful for the analysis of larger numbers of samples, such as from well-characterized patient biobanks, and contribute to the clinical as well as other omics data types.

**Methods**

We have developed and applied different types of highly multiplexed bead-based assays for discovery and validation of proteins in body fluids. Driven by the resource of reagents from the Human Protein Atlas ([www.proteinatlas.org](http://www.proteinatlas.org)), we applied antibodies and antigens to study body fluids from different disease areas. The conducted studies were designed with randomized samples to reduce unwanted bias, considering information about both the specimen and donor. Multidimensional data processing is then applied prior to statistical analysis. Combinations of using paired antibodies, epitope mapping, as we all as immuno-capture mass spectrometry (IC-MS) and genetic data is then chosen to confirm the discovered target protein and direct the development of sandwich assays for validation.

**Results**

We performed several systematic explorations of proteins profiles using antibody-based assays in 1,000s of plasma samples from longitudinal, clinical or case-control studies in health and disease. While discovery approaches are often fast and efficient, they demand complex validation schemes. Hence, we established workflows including IC-MS with plasma that enabled us to validate antibodies prior sandwich assay development and to describe protein complexes in plasma. We further observed that careful analysis of available clinical and sampling data is essential and found use in other omics as a guide towards successful target validation.

**Conclusions**

Post-discovery validation strategies and stringent study designs enable affinity assays to contribute with new candidates for personalized plasma proteomics. Examples from projects conducted within wellness and diseases will be given to illustrate the opportunities and challenges faced while translating discoveries into validated assays.

Keywords Plasma, study design, immuno-capture MS, validation, affinity assays