Designing sandwich assay workflows for large scale studies of plasma biobanks

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**Background:** Multiplex sandwich immunoassays are a very attractive approach for quantification of several proteins in parallel. In order to maintain the lead over other progressing proteomics approaches, there is a need to adjust the current assay formats and to meet the increasing number of samples collected in larger plasma biobanks. While the common 96 well assay format will remain useful, we have worked on accommodating a simple and accurate workflow to handle larger sample sets.

**Methods:** The optimization of the sample and assay workflow was performed within the EU IMI project DIRECT. Besides analyzing samples with Olink and ProteinSimple platforms, we used a 4-plex assay intended to profile more than 3100 plasma samples, collected from pre-diabetic and type II diabetic patients. Choosing magnetic beads and the FlexMap3D system, our aim was to build a procedure indented to be feasible for a single operator. Starting with reagents from four 96 well format assay kits, we designed a sample layout and compared the volume ratios for the 96 and 384 well assay format. To reduce hands-on-time and increase use of automation to address reproducibility concerns, a liquid handler (SELMA, CyBio) was used for parallel sample dilution and transfer from 96 well plates into one 384 well plate. Washing steps were performed using a plate washer (EL406, BioTek). We measured the consistency between the two assay formats and reagents volumes, and we studied the quantitative values obtained through the included standard rows.

**Results:** For thelarge-scale sample analysis, the transition from 96 into the 384 well format reduced the total number of batches from 36 to 9, enabling a turnaround of 3000 samples within two weeks’ time. For three analytes, 99.8% of the samples were above LLOQ. The fourth analyte was low abundant in our samples, making the dilution less optimal and 24.5% of the samples reached above LLOQ. The coefficient of variation between batches was calculated based on the mean of the pools within one batch. This was calculated for each of the four proteins, which varied between 7.3-11.3% depending on target protein.

**Conclusion:** We implemented a simple, upscaled workflow to perform 96 wells assays in the 384 well format and matched this design with other immunoassay platforms. With the anticipated four-fold decrease in batch numbers, this strategy also allowed us to reduce sample and reagent consumption. The workflow and plate design facilitated the use of auxiliary liquid- and bead-handling devices to reduce hands on times, hence enabled to bring current multiplex kits to a higher sample throughput.

**Keywords:** Protein profiling, FlexMap3D, assay optimization, 384-well assays, plasma