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Implementation of Experimental Design Techniques to Optimize Immunoglobulins detection with Simoa SP-X

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The measurement of ultralow levels of proteins plays a pivotal role in a number of disciplines, particularly in the diagnostic field. Indeed the early diagnosis of diseases through the ultrasensitive detection of protein biomarkers is of paramount importance [1]. In recent years, digital measurement methods such as Single Molecule Arrays (SiMoA) and digital Enzyme-Linked Immunosorbent Assay (ELISA) have made significant progress in terms of ultrasensitive protein detection with different clinically relevant biomarkers [2]. The SiMoA technology, unlike the conventional ELISA, is based on the high-density printing of capturing antibodies in each well of a microtiter plate. Next, the biotinylated detector antibody (in solution) forms a "sandwich" with a target antigen in between. The formation of the immunocomplex is detected through the reduction of H_2O_2 catalyzed in the presence of a chemiluminescent substrate by Streptavidin Horseradish Peroxidase (SA-HRP) bound to the biotinylated antibody [3]. While printing arrays of antibody has proven to be manufacturable, its uptake has been limited, as it requires highly optimized instrumentation and printing processes. To overcome this issue many laboratories use commercially available pre-rated and validated kits for specific biomarkers. Alternatively, Simoa Planar Array Homebrew kit is commercially available. It can be used to develop custom assays suitable for the immobilization of the desired capture antibody through a pair of peptide tags of an "anchor" antibodies printed on the well surface. A Simoa Planar Array Homebrew Kit for the detection and quantification of IgM, non-specific indicator of inflammation, has been developed and optimized. The experimental design has been undertaken to optimize the assay, leading to reduced experimental effort as well as increased quality of the information obtained with respect to the traditional 'one-variable-at-a-time (OVAT)' approach [4].

References

[1] L. Cohen and D. R. Walt, "Highly Sensitive and Multiplexed Protein Measurements," *Chemical Reviews*, **119**, 1, 293-321 (2019).

[2] C. I. Tobos, A. J. Sheehan, D. C. Duffy, and D. M. Rissin, "Customizable Multiplex Antibody Array Immunoassays with Attomolar Sensitivities," *Anal. Chem.*, **92**, 7, 5613-5619 (2020).

[3] C. I. Tobos *et al*., "Sensitivity and binding kinetics of an ultra-sensitive chemiluminescent enzyme-linked immunosorbent assay at arrays of antibodies," *J. Immunol. Methods*, **474**, (2019).

[4] R. Leardi, "Analytica Chimica Acta Experimental design in chemistry : A tutorial," *Europe*, **652**, 161–172 (2009).