xMAP® – An App to Analyze, Visualize, and Inspect Dilution-base Serological Assay Using the xMAP Technology

Stephan Michalik1, Tanja C. Meyer1, Frank Schmidt1, Uwe Völker1

1Department of Functional Genomics, Interfaculty Institute for Genetics and Functional Genomics, University Medicine and University Greifswald, Felix-Hausdorff-Straße 8, D-17475 Greifswald, Germany.

e-mail: stephan.michalik@uni-greifswal.de / tel.: +49 3834 420-5821

Background & aim:

The detection and quantification of human antibody levels directed against a specific antigen is of utmost importance in several fields of healthcare diagnostics, vaccine development, and research. The xMAP® technology allows a rapid cost-efficient high-throughput measurement of liquid biopsy antibody responses to multiple antigens with high sensitivity and specificity. Since the introduction of xMAP® technology serological analyses have usually been performed with one or at most two dilutions after prior estimation of the possible dynamic range of the included samples. However, the quantitative dynamic range of antibody levels can extend over several orders of magnitude and a more advanced dilution-based approach is needed to generate a high-dynamic range (HDR) data set without saturation issues.

Methods:

We introduce xMAP®, a browser-based app, which is written in R using the shiny package for an easy and intuitive GUI user experience. Shiny is a platform to build standalone web apps, and is compatible with a vast range of operating systems like Windows, MacOS and Linux.

After uploading the raw data into the pipeline of xMAP®, it filters every generated data point for the bead-region specific limit of detection (LOD = 3 x SD of blank value) and count (≥ 35 beads) as acquired in the assay and subsequently fits a saturation curve model for each antigen and sample. The model allows the extraction of one quantitative data point in the linear range, more precisely the half-maximal occupancy of the presented antigen by antibodies, of the individual dynamic range for each antigen/sample combination.

Several functionalities like PCA, heatmaps, signal antigen/sample combination plots for raw data inspection and group-wise Wilcox rank sum tests are part of the xMAP® app to name just a few implemented features.

Results:

The serial dilution serological assay analysis allows detection of antibody levels without limitations in the upper and much reduced lower detection limit. In a quantitative serological assay analysis with bacteremia patients, we could show that in a multiplex assay every chosen sample dilution bears detection limit issues, resulting in compromised quantitative data. Furthermore, the hook effect, which often occurs during the measurement of low diluted samples and high responding antigens could be explained by a lack of detection antibody not granting full saturation. The resulting signal drop in the data can be detected by the implemented signal-drop-detection-algorithm of xMAP® and exclusion of these values for down-stream analysis allows a highly accurate quantitative calculation of the antibody response level no matter what sample/antigen combinations were used in the multiplex assay.

Conclusion:

A serial dilution approach should be used when performing a multiplex serological assay analysis to avoid detection limit issues and to generate HDR data. xMAP® can deal with HDR data and guides the user from raw data up to a simple statistical analysis using its various functionalities. Quantitative data can be inspected globally and on the individual single antigen/sample combination level down to the raw data giving the user freedom and control in data inspection and interpretation. The implemented signal-drop-detection-algorithm deals efficiently with potential hook effects in the acquired serial dilution data.