Development and Analytical Validation of a Flexible Multiplexing Platform for Cytokine Assays

Pu Liu, Qian Ning, Sameera Rangwala, Chris Shelburne, Lori E. Kobayashi, Claire Lu, Eric Sandberg, David Stewart, Michael Tsionsky, Tatiana Plisova, Eli N. Glezer, Pankaj Oberoi, and Jacob N. Wohlstadtter

Meso Scale Discovery, Rockville, Maryland, USA

Abstract

Multiplex assays have advantages in being able to reduce time and sample volumes; however, multiplexing offers additional challenges, which include developing multiple assays that can be run with the same protocol, reagents, and sample dilution. Moreover, transferring reagents that work in one assay format to another without compromising performance and the integrity of sample measurement is difficult. The U-PLEX® platform enables flexible multiplexing of immunoassays using MSD’s MULTI-ARRAY™ technology. Antibodies used in V-PLEX® kits were transferred to the U-PLEX platform by biochemically attaching the capture antibodies. The assays represented a variety of analyte classes (chemokine, interleukin, interferon), antibody types (monoclonal, polyclonal), and analytical properties (sensitivity, dynamic range, concentration-response slope).

Methods

MSD’s electrochemiluminescence (ECL) detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microlites. The U-PLEX assay platform utilizes ten unique linkers that specifically bind to individual spots enabling simple and flexible creation of multiplex immunoassays. The table below demonstrates typical data for the first set of 14 biomarker assays tested. Data include Hill slopes and lower limits of detection (LLD) for the calibration curve. The calibrator is reconstituted and diluted serially (4-fold) to generate a 7-point standard curve.

Calibration Curves

The figures at right demonstrate typical calibration curves for 14 biomarkers. Up to ten markers can be combined per plate to create the desired multiplex. The specificity for each of the markers was evaluated by testing for cross-reactivity for each capture-detector pair with all 51 analytes included in the U-PLEX Biomarker Group 1 (human). No significant cross-reactivity was observed within the 51 human analytes from the group. Non-specific binding was less than 0.5% for all assays.

Reproducibility

Reproducibility of assays was evaluated by testing controls at three levels across the linear range of the calibration curve. The measured concentrations for six representative analytes are plotted in the graphs below. The table above shows the average recoveries. The results indicate that assays developed on the U-PLEX platform are highly reproducible.

Comparison with V-PLEX Assays

Fifty-one human serum and 51 EDTA plasma samples were tested with both U-PLEX and V-PLEX assays using the same reagents. The measured concentrations for six representative analytes are plotted in the graphs at right.

Summary and Conclusion

The U-PLEX technology successfully allows one to design and build multiplex biomarker panels on the sensitive MSD platform rapidly and in a few easy steps:

- Data for the optimization of 14 human biomarkers from the U-PLEX Biomarker Group 1 (human) are presented.
- Using the U-PLEX reagents, investigators can now combine novel biomarker assays using their own antibodies with assays included in the MSD menu. The antibody sets and calibrators provided in the menu have been optimized for the U-PLEX platform.
- The data generated on the U-PLEX platform correlate well with MSD’s validated V-PLEX kits.

Precision

Controls were made by spiking calibrator into assay diluent at three levels (high, mid, and low) within the quantitative linear range of the assay. The controls were measured using a minimum of two replicates tested over two days on nine runs and across three U-PLEX platform lots. Average intra-run %CV is the average %CV of the control replicates within an individual run. Intra-run %CV is the variability of controls across multiple runs. The %CV (concentration) for both inter- and intra-assay precision were found to be within acceptable limits (≤5%), and in most cases the intra-assay CV was less than 10%.

Spike Recovery

Normal human serum (n=15) and EDTA plasma (n=15) samples from a commercial source and cell culture media were spiked with calibrators at three levels (high, mid, and low). The percent recovery for most of the assays was within the acceptable range (70–130%).

Native Sample Testing

Normal human serum (n=10) and EDTA plasma (n=10) samples from a commercial source were tested neat. To demonstrate the detection of native analyte(s) in matrix, serum (n=5) and plasma (n=5) samples were spiked (spike volume ≤10%) with cell culture supernatants derived from peripheral blood mononuclear cells (PBMCs) that were stimulated with different compounds in vitro. These samples are depicted as spiked serum and EDTA plasma samples. Results for each sample set are displayed below.