Simple Plex™ Validated for Biomarker Analysis in Immuno-Oncology Studies

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Abstract

Designed to eliminate limitations of existing immunoassay platforms, Simple Plex™ assays utilize a low volume micro-fluidic cartridge format and are run in automated fashion on the Ella instrument, enabling sensitive measurement of up to 4 analytes in about an hour.

Here we describe recent external Immuno-Oncology studies evaluating Simple Plex assay performance for biomarker measurements from a range of sample types. Dynamic range, sensitivity, and reproducibility were all important test criteria in these studies evaluating dysregulation of pro-inflammatory cytokines and expression of immune checkpoint targets and downstream mediators.

Results demonstrated consistent sensitivity at low picogram/mL levels with 10% CV or less, a 4-5 log dynamic range across all sample types, and significant time savings versus traditional ELISA. Overall, Simple Plex assays enabled more sensitive detection in a quarter the time from a quarter the sample volume for measuring single or multiple analytes.

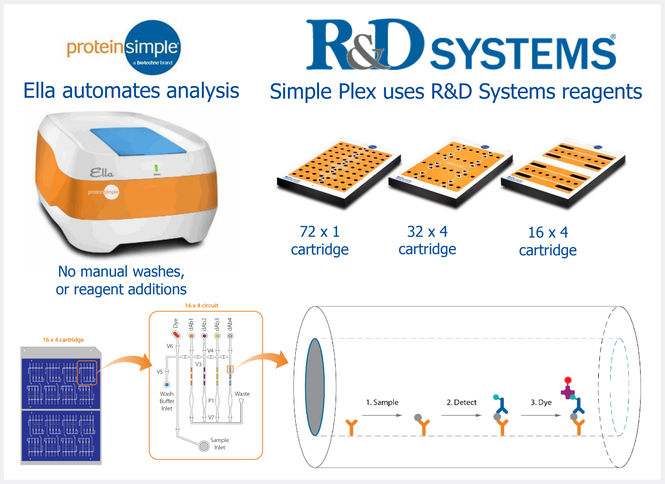

Figure 1. Simple Plex assays are automated sandwich immunoassays performed within analyte-specific glass nano reactors, or GNRs (right). A bottom view of the "16x4" multi-analyte cartridge is shown here (left). Each of the 16 samples is analyzed in an independent microfluidic circuit (middle). Reagent delivery is precisely controlled by pneumatically actuated valves (V1-V7) and pistons (P1). In each circuit, the sample is split into four parallel microfluidic channels, each with a dedicated well containing the detect antibody (dAb1-dAb4) corresponding to the triplicate GNRs in that channel. This spatial separation eliminates potential cross-reactivity from antibody pairs, while rapid microfluidic reaction kinetics ensure sensitive analyte detection from low sample volumes. Each analyte is measured by fluorescence quantitation of triplicate GNRs that are functionalized with capture antibody prior to being embedded in microfluidic channels.

Genentech – Reproducibility of Factory Calibrated Standard Curves

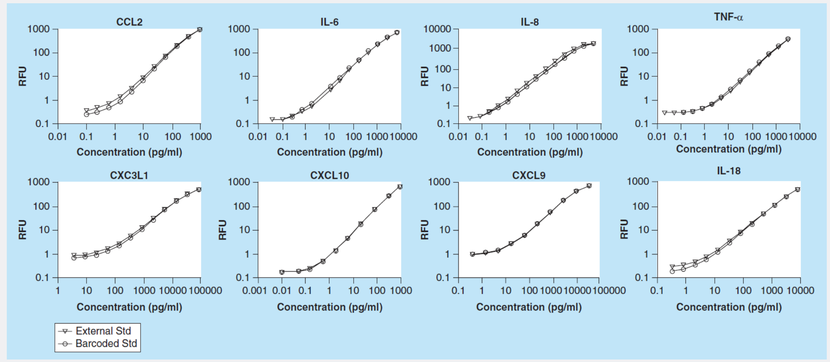

Figure 2. Overlay of Simple Plex "factory calibrated" barcode embedded standard curves compared to external standard curves generated at Genentech. High consistency reported for all 8 biomarkers tested in this study.

At Genentech, bioanalytical researchers Vinita Gupta, Teresa Davancaze and Jeremy Good performed fit-for-purpose bioanalytical qualification of Simple Plex assays. As shown in Figure 2 above, both factory provided and external standard curves for all eight markers demonstrated consistent high sensitivity and broad dynamic range. This accurate and consistent biomarker quantitation qualified Simple Plex for usage in future mechanistic studies of immune checkpoint inhibitors. **(Reference: V Gupta, T Davancaze, J Good, et al, Bioanalysis (2016), 8(23), 2415-2428.)**

Sensitivity & Range Enable Biomarker Quantitation in Healthy and Disease Samples

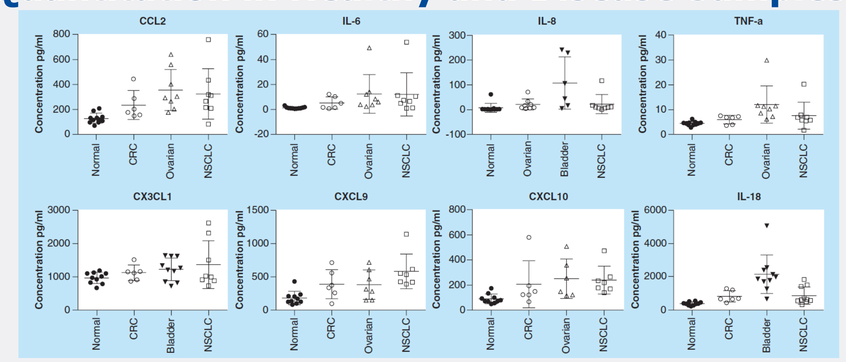

Figure 3. Simple Plex sensitivity and dynamic range enables quantitation of all 8 biomarkers in both normal healthy and cancer patient samples. Plots show calculated analyte concentrations in apparently healthy donors (n = 9–10), colo-rectal cancer (CRC, n = 6), ovarian cancer (n = 7), NSCLC (n = 7–8) and bladder cancer (n = 6–10) patients.

At Genentech, plasma biomarkers were measurable at low levels in normal, healthy reference samples as well as at higher levels found in disease state samples. Conclusion: "Simple Plex met the needs for sensitivity and specificity." **(Reference: V Gupta, T Davancaze, J Good, et al, Bioanalysis (2016), 8(23), 2415-2428.)**

NIH - Ella Generates Better Data Faster

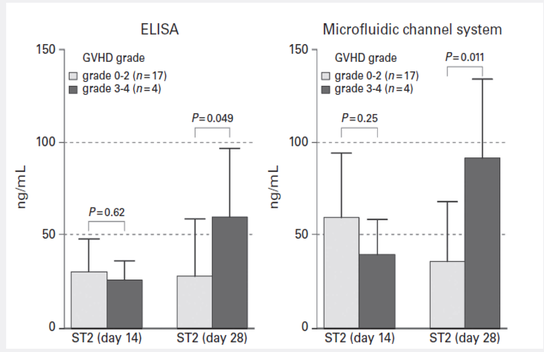

Figure 4. Simple Plex demonstrated better reproducibility and produced more rapid results compared to ELISA for GVHD monitoring application.

AJ Barrett's lab at NIH used their Ella instrument to study transplantation rejection (Graft versus Host Disease – GvHD) that can result from stem cell transplantation for hematological malignancies. As shown in Figure 4, both ELISA and Simple Plex (listed as 'Microfluidic channel system') detected increases in ST2 levels between day 14 and day 28 that were associated with severity of GvHD. However, Simple Plex multi-analyte analysis of four biomarkers showed better assay reproducibility and could be performed in far less total time (1-2 hours versus 16 hours) than single analyte ELISAs. **(Reference: P Anandi, A J Barrett, S Ito et al, *Bone Marrow Transplantation* (2016), 51, 1615-1616.)**

Quantikine Correlated & More Sensitive

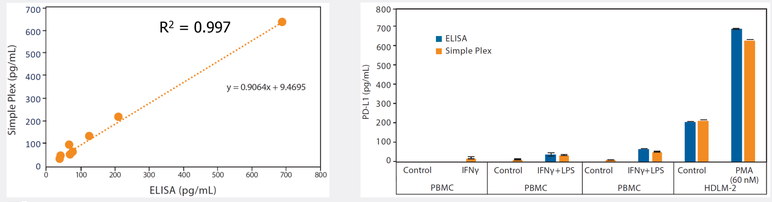

Figure 5. Consistent PD-L1 measurements between Quantikine ELISA and Simple Plex (left). Sensitivity enables Simple Plex quantitation of low basal PD-L1 levels as well as higher induced levels in stimulated cell supernatant (right).

Measured levels of PD-L1 in glioma cell supernatant, PBMC, and HDLM-2 samples correlated very well between Simple Plex and ELISA assays (R^2 correlation coefficient of 0.997). **(Reference: ProteinSimple Application Note (2016) - High Fidelity Detection of Endogenous PD-L1 at Low Picogram Levels with Simple Plex Assays)**

Conclusions

Simple Plex™ assays deliver sensitive, quantitative, and reproducible biomarker measurements from low volumes of multiple sample types.

- Consistent sensitivity and reproducibility– low pg/mL results, < 10% CVs
- Wide dynamic range (4-5 log) for quantitation of control & test samples
- External validation of barcoded factory calibration standard curves
- Exceptional ease of use – no manual washes, built-in triplicates
- Automated Ella system minimizes variability, provides rapid results