Detecting and Analyzing Cytokines the Simple Plex Way

Fast and Reproducible Quantitation of Cytokines with Ella

Introduction

As small soluble protein messengers released by cells, cytokines interact and communicate with other nearby cells. The results are pleiotropic and affect cellular function in ways that can indicate inflammation or disease progression, making cytokine detection, measurement and monitoring important to clinicians and researchers alike. The enzyme-linked immunosorbent assay (ELISA) is the traditional way to detect and validate protein biomarkers in serum, cell preparations or other biological sample types because the assay offers high target specificity.

However, the standard ELISA approach doesn't always have the sensitivity or reproducibility required to detect endogenous levels of an analyte reliably. Moreover, analyte cross-reactivity complicates multiplexing, and the tedious manual workflow that calls for hours of time to produce a result followed by hours of time for analysis has necessitated change and produced an improved, alternative approach that streamlines the entire process. In this application note, we'll show you how scientists are already using Simple Plex™ assays on Ella™ to do everything an ELISA does, but faster. Oh, and with greater sensitivity and reproducibility.

What is the Simple Plex Way?

All steps of a Simple Plex immunoassay on Ella are highly automated thanks to her microfluidic cartridge—everything is preloaded, even the calibration curve. Just pipette your diluted samples onto the cartridge, add wash buffer, press Start in the Ella Runner Software and walk away to the sound of automatic processing. Inside the Simple Plex cartridge, samples are routed through microfluidic channels containing Glass Nano Reactors (GNRs) (**Figure 1**). Each GNR is coated with an analyte-specific and pre-optimized capture antibody. With three GNRs in each microfluidic channel, you automatically get triplicate results for every analyte. Setup only takes 10–15 minutes, and results are ready in about 1 hour. With the Simple Plex workflow, you'll have low-pg/mL detection and single-digit inter- and intra-assay coefficient of variation (CV) values giving you superior assay sensitivity and data reproducibility.

From the Field: Using Simple Plex Assays to Detect Cytokines

In all areas of life science research, the peer review process is, in more ways than one, a hurdle to your success. This is especially true with regard to cytokine immunoassays, where scientists are regularly challenged on the reproducibility and variability of results. We just told you how Ella works and why she is your immunoassay problem solver; now, we're including a selection of peer-reviewed work for that extra seal of external approval you'd expect.

CHARACTERIZATION AND VALIDATION OF SIMPLE PLEX

Researchers at Yale University School of Medicine and collaborating colleagues used Simple Plex assays to quantitatively measure cytokine levels in various human and mouse sample types, including tissue, serum, plasma, cell lysate and supernatant[1]. To examine inter-assay

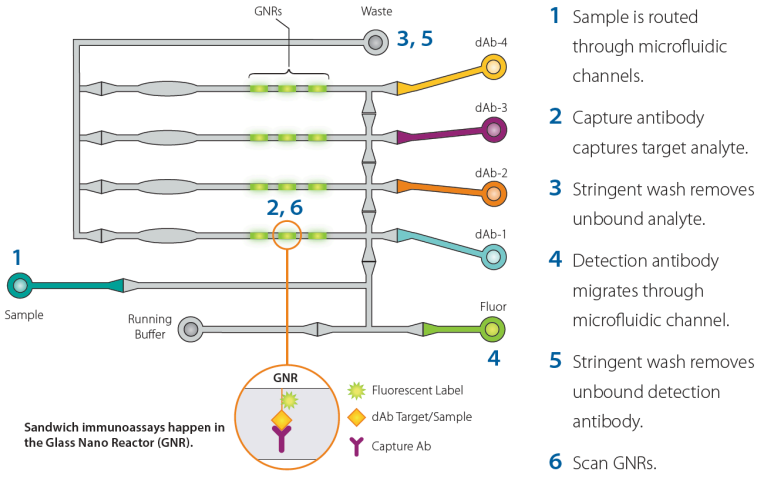

FIGURE 1. Ella performs immunoassays in a microfluidic Simple Plex cartridge. The sample is first routed through the microfluidic channels, then a capture antibody captures the target analyte. Stringent washes follow, which remove unbound analyte. Detection antibody migrates through the microfluidic channel, while stringent washes remove any that is unbound. Finally, GNRs are scanned for further data analysis.

reproducibility, samples were run by two analysts using separate Ella instruments and at independent laboratory sites. The team measured the concentration levels of four cytokines—IL1-b, IL-5, IL-10 and IL-6—in 15 individual human serum and plasma samples in duplicate. To generate standard curves that are independent of being factory-calibrated, samples were run blind, each spiked at low, medium, and high concentrations, and analyzed using data from the two sites/users. The analysis demonstrated a strong linear relationship ($R^2 > 0.98$) between the sites, instruments and users for all sample types and analytes tested (**Figure 2A**). To further attest to assay reproducibility, the team generated 11 standard curves for the cytokine IL-6 over 31 days, for which a representative curve is shown in **Figure 2B.** With a CV of <5%, the researchers were able to conclude that Simple Plex assays have "a high level of precision and reproducibility" and that the "resultant calculated concentrations also demonstrated excellent reproducibility"[1]. Finally, to address inter-assay precision, they ran high- and low-quality control values of the four analytes under study and determined excellent reproducibility across different cartridges over a multi-day period (**Figure 2C**).

In summary, this work both characterizes and validates Ella for quantitative and reproducible analyses of cytokines in human and mouse samples. It also shows the excellent transferability of a Simple Plex assay. Multiple users at different sites were able to leverage Simple Plex technology to get the same high-quality cytokine measurements. This gives you the peace of mind that your work can be easily transferred to collaborators, or into publication.

MULTIPLEX WITH SENSITIVITY

The work published in the high-impact journal *Cell* by researchers in The Netherlands attests to the highly sensitive detection capabilities of the Ella platform[2]. Therein, Simple Plex assays are used to assess cytokine levels in the fg/mL to low pg/mL concentration range! To examine various environmental and non-genetic host factors that alter the immune response, Rob ter Horst and colleagues use a systematic approach including over 500 healthy subjects within a broad age range to measure the production and release of cytokines in response to bacterial, fungal, viral and nonmicrobial metabolic stimuli.

Initially unable to measure resting levels of cytokines in healthy individuals due to their low abundance, the team turned to Simple Plex assays on Ella to obtain this baseline. Because Simple Plex assays have a wide dynamic range—3 to 5 logs—which is typically 1 to 2 logs greater than a standard ELISA, researchers could detect IL-1β, IL-6, IL-18 and VEGF in the fg/mL to low pg/mL range[2]. This ability allowed for the comprehensive analysis of circulating cytokines and facilitated the reported correlation between cytokine production and age and gender in healthy individuals. Moreover, in an ex-vivo system, cytokine expression monitoring after exposure to microbial stimuli

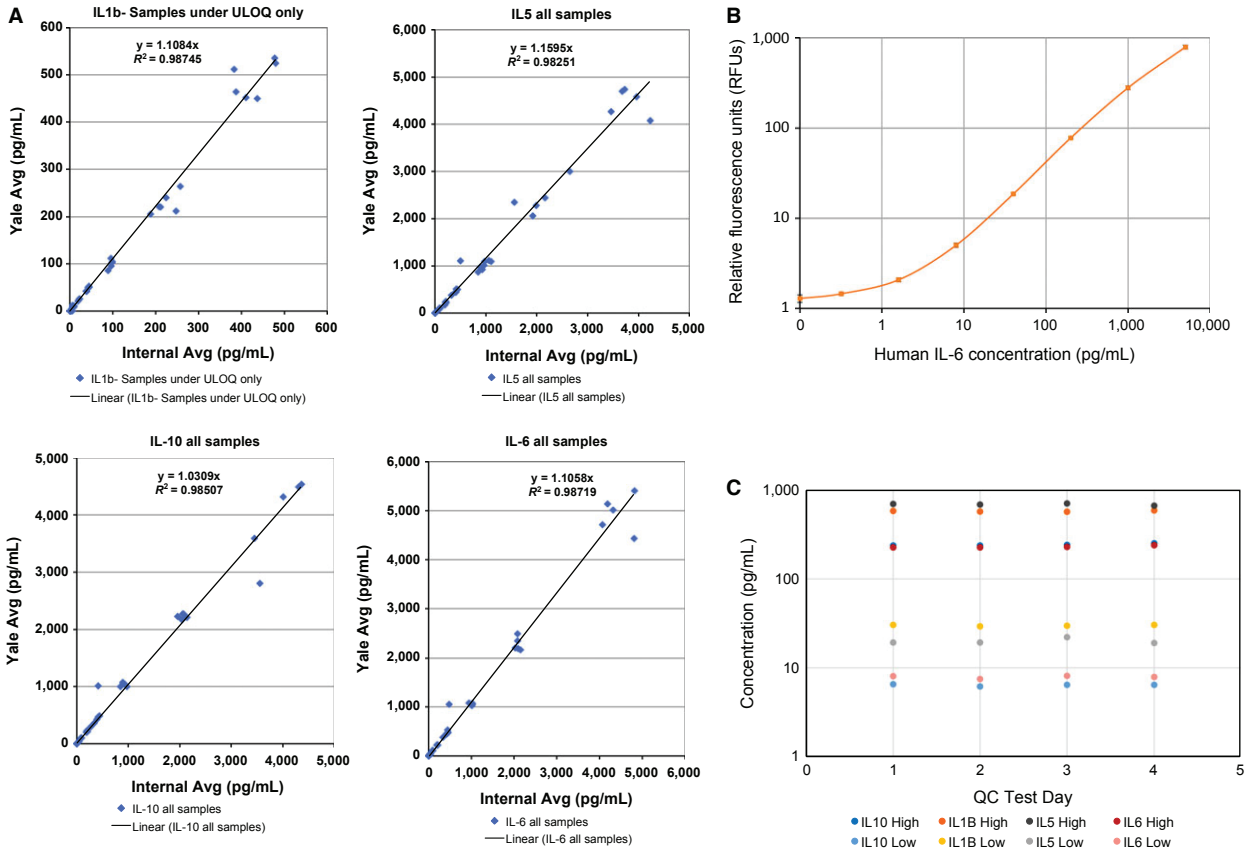

FIGURE 2. Sample Reproducibility: A standard curve for analytes was generated by two separate laboratories at different times using the same batch of panels. Representative curves for four analytes (A). **Standard Curve Reproducibility:** Stability and reproducibility of the assay were established by generating 11 individual standard curves for human IL-6 run over the course of 31 days (B). **Intra-assay Precision:** Intra-assay precision was determined for each analyte (IL-10, IL-1b, IL-6 and IL-5) by running cartridges containing eight replicates of either high or low QCs over a period of 4 days (C). Adapted from Aldo *et al.*, 2016, CC BY 4.0[1].

also resulted in differences that could be attributed to age, gender and seasonality. This work has important implications in our understanding of how variations in the immune response could influence one's susceptibility to a number of autoimmune diseases and other pathologies.

LEVERAGE IMMUNODETECTION DATA

The ability to measure several proteins at once in any given cellular or bodily fluid preparation provides researchers with a more sophisticated view of their biology while saving on both sample volume and time spent. This has been doable using other immunodetection-based techniques such as Western blotting (due to size differentiation) but is otherwise a struggle using traditional ELISA.

Working with primary human nucleus pulposus cells to investigate the impact of pH on the inflammasome-regulated inflammatory response, researchers in the Department of Neurological Surgery at the University of Miami used Simple Plex assays to study the role of cytokines IL-1β and IL-18 in lower back pathology[3]. A representative immunoblot and the associated densitometry analysis (**Figure 3 A-C**) shows significant changes in intracellular IL-1β and IL-18 protein expression in response to decreasing pH levels. This result is further strengthened by the quantitative results obtained from the Simple Plex assays (**Figure 3 D and E**), which show the concentration of released extracellular protein also being meaningfully reduced at a pathological pH ($p < 0.05$, $n = 5–6$ wells/group run in triplicates). With Ella, researchers were able to leverage the protein expression information gained by traditional immunoblotting to provide confirmatory results for their initial observation, paving the way for the mechanistic studies that follow.

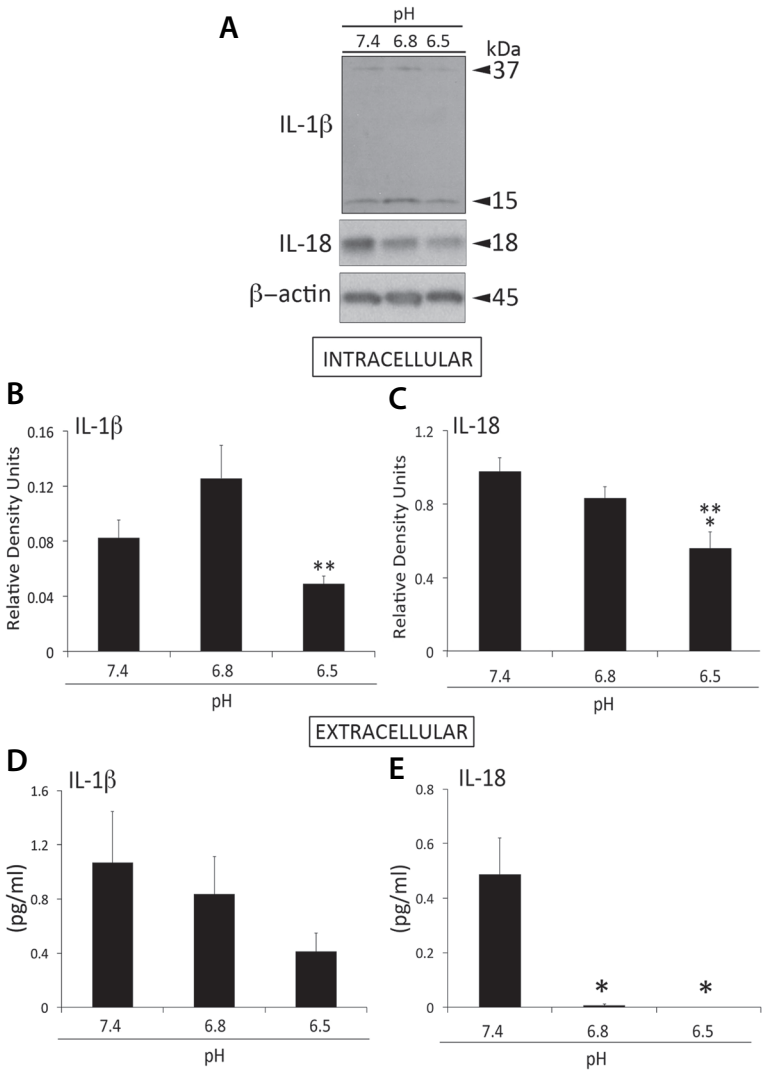

FIGURE 3. IL-1 cytokine expression is reduced at pathological pH. Representative immunoblot analysis of IL-1β and IL-18 at three different pH levels (7.4, 6.8 and 6.5) (A). Densitometric analysis corresponds to the active forms of IL-1β (15 kDa, B) and IL-18 (18 kDa, C). Data presented as mean ± SEM. N = 6 wells per group. β-actin was used as an internal standard for protein loading control. Cell media were used to measure protein concentration of released IL-1β (D) and IL-18 (E). Data presented as mean ± SEM. N = 5–6 wells per group. **$p < 0.05$ compared to 6.8 and *$p < 0.05$ compared to 7.4. Data on A, B and C were obtained from lysates measured by immunoblotting, and data from D to E correspond to supernatants measured by a Simple Plex assay. Adapted from Brand III *et al.*, 2016, CC BY 4.0[3].

Conclusion

Ella will transform the way you measure cytokines. She offers a fast, robust method for single- or multi-analyte quantitation in biological matrices. With a fully automated workflow, the protocol challenges that come with performing traditional ELISAs are eliminated. In this application note, we've shown you some of the ways in which researchers are using Ella to generate highly precise and quantitative cytokine data—all without any of the hassle and in just 1 hour.

References

1. Simple Plex™: A novel multi-analyte, automated microfluidic immunoassay platform for the detection of human and mouse cytokines and chemokines, P Aldo, G Marusov, D Svancara, J David, and G Mor ***American Journal of Reproductive Immunology***

2. Host and environmental factors influencing individual human cytokine responses, R Horst, M Jaeger, SP Smeekens, M Oosting, MA Swertz, Y Li, V Kumar, DA Diavatopoulos, AFM Jansen, H Lemmers, H Toenhake-Dijkstra, AE van Herwaarden, M Janssen, RG van der Molen, I Joosten, FCGJ Sweep, JW Smit, RT Netea-Maier, MMJF Koenders, RJ Xavier, JWM van der Meer, CA Dinarello, N Pavelka, C Wijmenga, RA Notebaart, LAB Joosten, and MG Netea, ***Cell***, 2016; 167:1111–24.

3. Acidification changes affect the inflammasome in human nucleus pulposus cells, FJ Brand III, M Forouzandeh, H Kaur, F Travascio and JP de Rivero Vaccari, ***Journal of Inflammation***, 2016; 13:29.

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