Ultrasensitive Measurements of Multiple Cytokines at the Single Molecule Level

Abstract

Demonstrated use of Simoa™ technology to measure cytokines in both singleplex and multiplex configurations with excellent precision and limits of detection in the low fg/mL or sub–fg/mL levels, allowing robust quantification well below what is possible today.

Introduction

Cytokines serve as central communicators for the immune system. Maintaining the delicate balance in the level of these proteins is essential for overall health. As mediators of the inflammatory response, these small protein molecules freely diffuse in systemic circulation and transmit signals to other cells. This balance is disrupted in many chronic inflammatory diseases, including rheumatoid arthritis, Crohn’s disease (CD) and ulcerative colitis. Chronic inflammation has been attributed to the increased production of cytokines that are responsible for triggering inflammation and development of the acute phase response.1 Highly successful therapies for treating chronic inflammatory diseases have been developed that target cytokine pathways and offer significant hope for patients. The use of more disease-specific, quantitative measures that accurately reflect disease severity and patient response to therapy would have several advantages over current methods.

The precise and accurate measurement of circulating cytokines is important for understanding their influence on disease pathogenesis, treatment, and prognosis. However, cytokine concentrations in healthy individuals and individuals with chronic inflammatory diseases are often at low single-digit pg/mL or sub–pg/mL levels.2 As physiological concentrations are close to or below the limit of detection of conventional technologies, not only will many samples go undetected, but also those that are measurable will have inherently high imprecision. As a result, it has proven difficult or impossible to accurately distinguish groups of patients or to monitor changes in cytokine concentration after administration of drugs or candidate drugs using assays with pg/mL sensitivity. Cytokine concentrations have, therefore, been of limited diagnostic utility.

For the measurement of cytokine concentrations to eventually be clinically useful, more sensitive assays are clearly needed. Quanterix™ has developed a novel platform that enables the quantification of proteins present in blood and other body fluids at very low, previously undetectable concentrations. The application of this Single Molecule Array (Simoa) technology will provide medical researchers with an unparalleled tool for detecting low-abundance biomarkers and help facilitate the development of a new generation of diagnostic products useful for early detection or treatment of disease.2 Unprecedented analytical sensitivity is the key differentiator of Quanterix’s technology, made possible by its proprietary single molecule detection (to learn more about Simoa, see whitepaper 1.0).

Study

Simoa was used to precisely measure two key cytokines, TNFα and IL-6, in the blood of patients with CD.4 With fg/mL sensitivity, it was possible to precisely measure these two proteins in the blood of all patients, and to monitor changes in concentration with treatment with approved antibody therapeutics. The analytical performance of these assays was evaluated and used to determine the concentration of these cytokines in patients before and after a 12-week course of anti-TNFα therapy (Fig. 1). Plasma TNFα concentrations were reduced by antibody treatment by an average

Figure 1  Plot of the concentration of TNFα in the plasma of 9 CD patients before treatment with anti-TNFα therapy (white bars) and 12 weeks after therapy was initiated (gray bars). With the exception of case 19, patients were anti-TNFα naive.

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of 48% for the nine patients measured in this study. Similarly, plasma IL-6 concentrations were reduced during therapy in the majority of patients tested. Previous studies have been unable to demonstrate a clear reduction in cytokine levels in blood because of insufficient assay sensitivity. Furthermore, TNFa levels in CD patients and healthy individuals were below the limits of detection of the most commonly used assays.

The improved sensitivity and precision of digital ELISA will enable useful measurements of individual cytokines in blood. However, the ability to minimize sample volumes, to eliminate the need to run multiple assays, and to precisely measure multiple proteins simultaneously is important in several fields, including clinical diagnostics. To this end, multiplexed Simoa is being used to measure the concentrations of up to 10 low-abundance proteins simultaneously. Figure 2 illustrates a 4-plex cytokine assay for TNFa, IL-6, IL-1a, and IL-1B that is capable of maintaining sensitivity comparable to individual singleplex assays down to low fg/mL detection limits. The corresponding protein measurements from normal healthy individuals were also measured at levels below the detection limits of conventional ELISA-based technology (Fig. 3).

**Conclusion**

Simoa has the potential to markedly improve our understanding of inflammatory diseases, including CD. Quanterix is developing a broad menu of individual and multiplexed cytokine assays to address unmet needs in the life science research market, including IL-2, IL-4, IL-5, IL-6, IL-8, IL-13, IL-17a, IFNy, and TNFa. For a complete listing of available assays, please visit www.quanterix.com.

**References**


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![Figure 2](image2.png)  
**Figure 2**  Plots of AEB against protein concentration for four cytokines measured in bovine serum samples spiked with all four cytokines. AEB is the Simoa unit of measurement that is used to calculate concentration.

![Figure 3](image3.png)  
**Figure 3**  Fifteen normal serum samples measured using the Simoa 4-plex assay.