# Quantitative Multi-Analyte Detection of Key Cytokine Release Syndrome Biomarkers:
## Workflow Optimization with an Automated, Microfluidic Immunoassay System

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## Abstract

We designed a quantitative multi-analyte detection assay of key cytokine release syndrome (CRS) biomarkers. This microfluidic parallel immunoassay measured four cytokines simultaneously in only one hour. We compared cytokine measurements for human IL-6, IL-1β, TNF-α, and IFN-γ between our Quantikine® ELISA and Simple Plex™ immunoassays using an *in vitro* model for CRS. Samples from all donors used in this *in vitro* model showed similar results across immunoassay platforms. The large dynamic ranges of the Simple Plex assays allowed for monitoring of baseline sub-picogram cytokine levels in untreated samples. This automated Simple Plex assay panel optimizes lab workflow from screening to precise detection of cytokines associated with CRS and other processes where monitoring immune responses accurately and quickly is essential.

## Introduction

Cancer immunotherapies involving monoclonal antibodies or adoptive T cell transfers are associated with an adverse reaction known as cytokine release syndrome (CRS). The pro-inflammatory cytokines produced largely by T cells and macrophages are prominent mediators of CRS[1-5]. Monitoring both the toxicity and efficacy of immunotherapy is essential for screening and proper management of these emerging cancer treatments. Our Simple Plex™ assay provides an easy solution by allowing custom-made, simultaneous measurements of pro-inflammatory cytokines IL-6, IL-1β, IFN-γ, and TNF-α with minimal sample volume and hands-on time. We developed an *in vitro* CRS model that uses high density precultured PBMCs, untreated or activated with immobilized anti-CD3ε and soluble antibody to CD28 (TGN1412, produced in-house). PBMC supernates from the CRS model were compared on our assay platforms. Our data show that quantification of CRS-related cytokines, using either Quantikine® ELISA or Simple Plex™ immunoassays, result in comparable measurements and reliability across a wide range of concentrations and donor variability. The larger dynamic range and multi-analyte capabilities of the Simple Plex™ platform make it an ideal solution for workflow optimization when monitoring immune responses in clinical or research settings without compromising the quality expected from Bio-Techne products.

## Methods

### Cell Culture

Human peripheral blood mononuclear cells (PBMCs) were cultured in RPMI supplemented with 10% FBS containing 2mM L-glutamine, 100 units/mL penicillin, 100 µg/mL streptomycin, and maintained in a 5% $CO_2$ incubator at 37 °C. PBMCs were isolated from the venous blood of four different donors via Ficoll-Paque PLUS™ (GE Healthcare, Cat# 17144002) density centrifugation. PBMCs were then pre-cultured at 1 x $10^7$/mL for 48 hours prior to culturing them at 1 x $10^6$/mL with or without immobilized mouse anti-human CD3ε (R&D Systems, Catalog # MAB100) and soluble humanized anti-human CD28 (TGN1412) for 48 hours before collecting and assaying cell supernates.

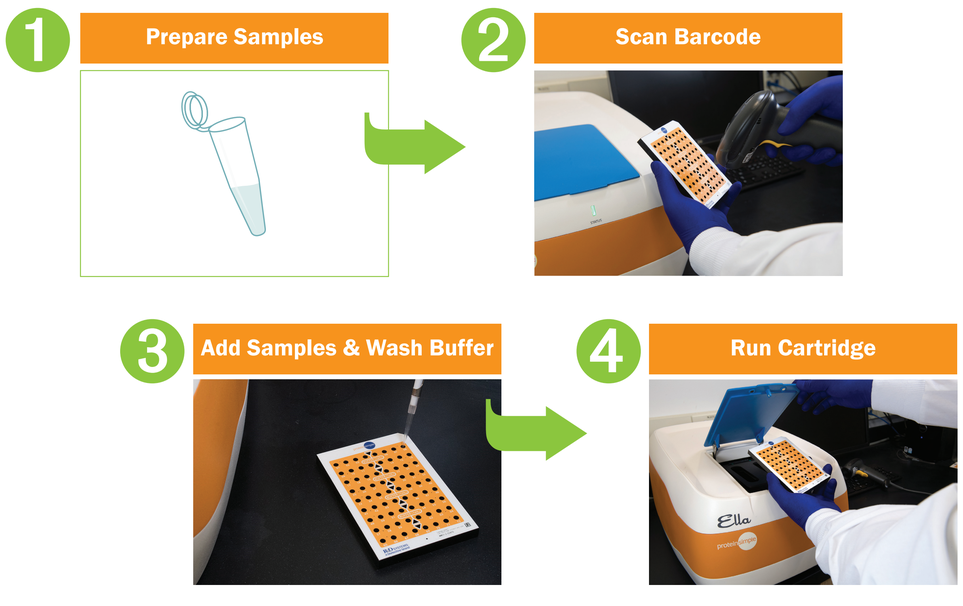
### Quantikine® ELISA Analysis

Cell culture supernates were analyzed on the Human IL-6 Quantikine® ELISA Kit (R&D Systems, Catalog # D6050), Human IL-1β/IL-1F2 Quantikine® ELISA Kit (R&D Systems, Catalog # DLB50), Human IFN-γ Quantikine® ELISA Kit (R&D Systems, Catalog # DIF50), and Human TNF-α Quantikine® ELISA Kit (R&D Systems, Catalog # DTA00C). Cell culture supernates were analyzed according to the procedures outlined in the product inserts. 100–200 µL of sample was required for duplicate data points in each kit.

### Simple Plex™ Assay

Cell culture supernates were analyzed on the Simple Plex™ 16 x 4 multi-analyte assay cartridge specific for human IL-6, IL-1β, IFN-γ, and TNF-α (ProteinSimple Product Code # SPCKA-PS-000290). Cartridges come with factory-set predetermined standard curves that are uploaded upon scanning the QR code. The procedure is quick, user-friendly, and requires 30 µL of sample to provide triplicate data points for each of the four analytes.

## Ella Workflow




## Simple Plex™ Recovery

### Table 1.

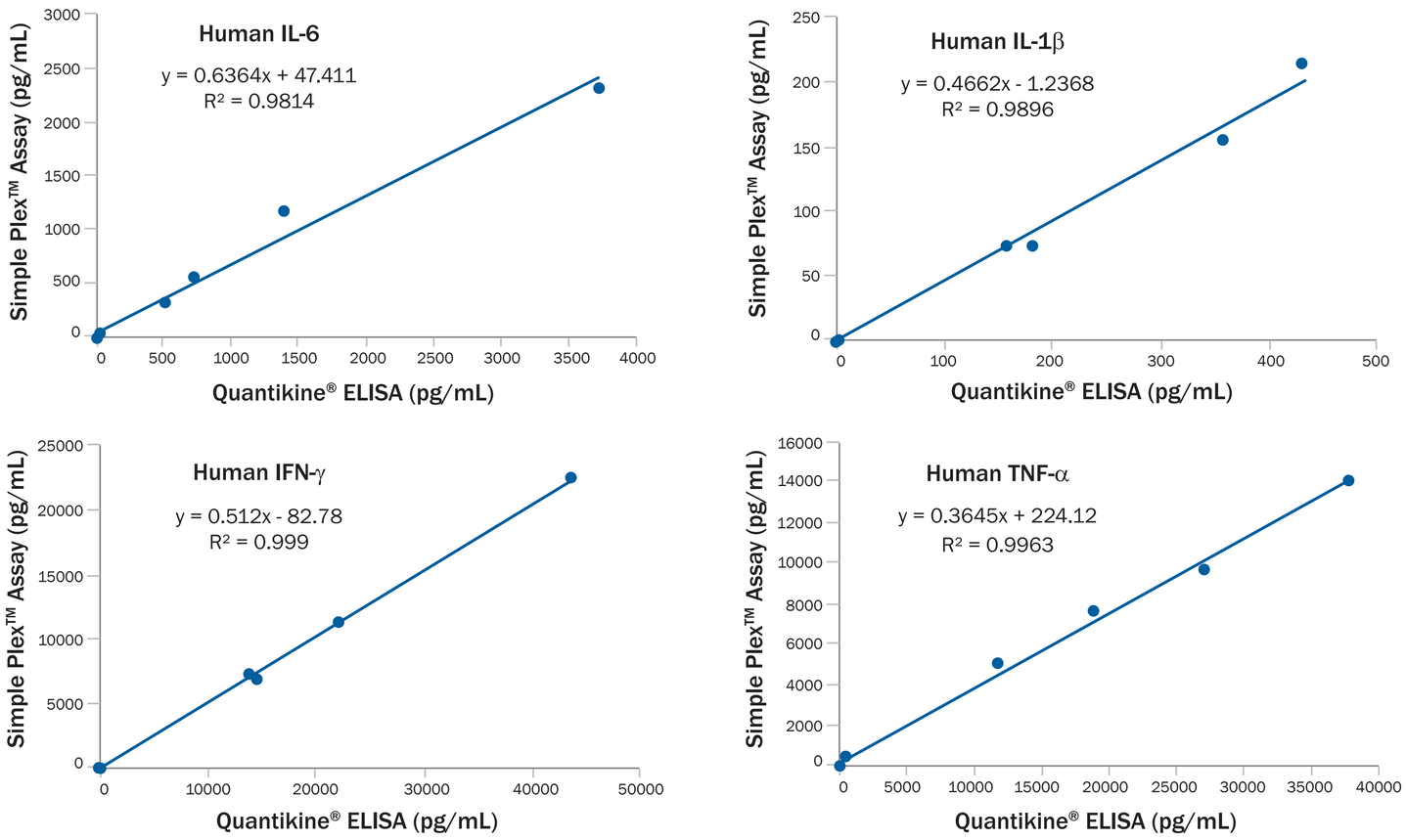
| | Sample Type | AVG % | RANGE % |
|---|---|---|---|
| Human IL-6 | Serum (n=6) | 88 | 74–98 |
| | EDTA Plasma (n=6) | 99 | 88–112 |
| | Heparin Plasma (n=6) | 85 | 70–105 |
| Human IL-1β | Serum (n=4) | 105 | 96–112 |
| | EDTA Plasma (n=4) | 106 | 97–120 |
| | Heparin Plasma (n=4) | 99 | 92–112 |
| Human IFN-γ | Serum (n=4) | 88 | 82–96 |
| | EDTA Plasma (n=4) | 87 | 80–90 |
| | Heparin Plasma (n=4) | 87 | 79–96 |
| Human TNF-α | Serum (n=4) | 86 | 77–95 |
| | EDTA Plasma (n=4) | 88 | 82–99 |
| | Heparin Plasma (n=4) | 87 | 80–93 |

**Recovery of human cytokines IL-6, IL-1β, IFN-γ, and TNF-α in Simple Plex™ assays.** Recombinant human IL-6, IL-1β, IFN-γ, or TNF-α was spiked into serum, EDTA plasma, and Heparin plasma at three different levels within the range of their respective assay and evaluated. Simple Plex™ assays have the same requirements for sample recovery as Quantikine® ELISA kits.

### Table 2.

| Sample | | IL-6 (pg/mL) | | IL-1β (pg/mL) | | IFN-γ (pg/mL) | | TNF-α (pg/mL) | |
|---|---|---|---|---|---|---|---|---|---|
| | | Quantikine® ELISA | Simple Plex™ Assay | Quantikine® ELISA | Simple Plex™ Assay | Quantikine® ELISA | Simple Plex™ Assay | Quantikine® ELISA | Simple Plex™ Assay |
| PBMC Donor 1 | Control | 27 | 23 | 0 | 1 | 0 | 2 | 79 | 97 |
| | Activated | 3,725 | 2,321 | 358 | 154 | 22,084 | 11,170 | 11,763 | 4,871 |
| PBMC Donor 2 | Control | 14 | 14 | 0 | 1 | 0 | 6 | 287 | 236 |
| | Activated | 1,374 | 1,180 | 432 | 215 | 43,430 | 22,342 | 37,705 | 13,929 |
| PBMC Donor 3 | Control | 13 | 15 | 0 | 0 | 0 | 1 | 68 | 78 |
| | Activated | 716 | 555 | 160 | 71 | 13,850 | 7,125 | 18,748 | 7,613 |
| PBMC Donor 4 | Control | 20 | 24 | 0 | 2 | 0 | 6 | 429 | 411 |
| | Activated | 516 | 324 | 180 | 72 | 14,358 | 6,674 | 27,008 | 9,581 |
| Standard Curve | | 3.13–300 pg/mL | 0.7–2,652 pg/mL | 3.9–250 pg/mL | 0.16–1,530 pg/mL | 15.6–1,000 pg/mL | 0.99–9,410 pg/mL | 15.6–1,000 pg/mL | 0.76–2,900 pg/mL |

**Quantitative analysis of activated PBMC supernates using an *in vitro* cytokine release syndrome (CRS) model.** Human PBMCs isolated from four separate donors were left untreated or activated using antibodies against human CD3ε and CD28 for 48 hours. PBMC supernates were collected and evaluated using Quantikine® ELISA kits from R&D Systems and Simple Plex™ 16 x 4 multi-analyte assay cartridges from ProteinSimple. Activated PBMCs from each donor had variable amounts of pro-inflammatory cytokines detected over a wide range of concentrations.




**Platform correlation data.** Pro-inflammatory cytokine levels from our *in vitro* PBMC supernates showed correlation between Quantikine® ELISA and Simple Plex™ assays. Correlation coefficients for human IL-6, IL-1β, IFN-γ, and TNF-α were >0.98 indicating excellent correlation between the two platforms. Simple Plex™ assays are correlated to their respective Quantikine® ELISA during development.

## Summary

- Key pro-inflammatory cytokines were detectable in PBMC supernates of all donors used for the *in vitro* CRS model
- There is a >98% correlation between the data generated utilizing Quantikine® ELISA and Simple Plex™ assays
- The custom multiplexing and sub-picogram sensitivity of the automated Simple Plex™ assay provides a streamlined workflow for monitoring immune responses

Taken together our data indicate that we can reliably measure CRS-related cytokines in a multi-analyte format that has small sample volume requirements and is completed in one hour. Importantly, Simple Plex™ assays are very sensitive and have a large dynamic range while still demonstrating comparable results with >98% positive correlation with Quantikine® ELISAs. In conclusion, Bio-Techne is able to provide the optimal assay solutions for your immuno-oncology needs.

## References

1. Suntharalingam, G. *et al.* (2006) N. Engl. J. Med. **335**:1018.
2. Maude, S.L. *et al.* (2014) Cancer J. **20**:119.
3. Römer, P.S. *et al.* (2011) Blood **118**:6772.
4. Findlay, L. *et al.* (2010) J. Immunol. Methods **352**:1.
5. Stebbings, R. *et al.* (2013) J Immunotoxicol. **10**:75.