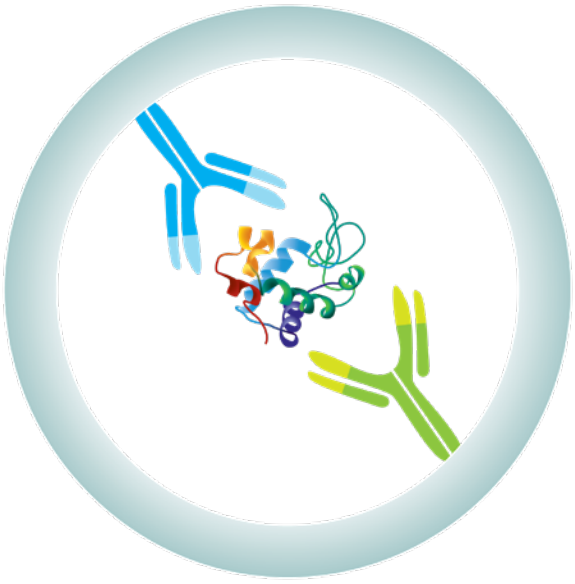
APPLICATION NOTE

Analyzing Crohn's Disease Biomarkers Utilizing Bio-Techne's Immunoassay Platforms: From Multiplex Discovery on Luminex® to Single Analyte Analysis with Quantikine® ELISAs

INTRODUCTION



Crohn's disease is an inflammatory bowel disease (IBD) that affects an estimated 1.6 million people in the United States alone according to the Crohn's and Colitis Foundation of America (CCFA). It primarily affects the gastrointestinal system and has long been recognized as a formidable disease with high recurrence rates even after surgical treatment is attempted. Anastomotic recurrence occurs endoscopically as early as several months after bowel resection, followed by symptomatic recurrence; reoperation is then often required.

Multiple clinical biomarkers of Crohn's Disease have been studied and developed into tests like serum C-Reactive Protein (CRP) and fecal calprotectin (FCP). Although there have been some studies to correlate biomarkers with endoscopic scores, relapses, and postoperative recurrence prediction, their main value thus far is in managing patients with Crohn's disease. Therefore, identifying novel, accurate circulating biomarkers is a research priority.

R&D Systems, a Bio-Techne Brand, offers a variety of specific and reproducible immunoassay platforms that make it easy to discover, analyze, and verify novel biomarkers and automate your favorite biomarker research. In this application note, we take you through a proof-of-concept example of how to streamline your biomarker studies by using R&D Systems' Luminex® assays for your biomarker discovery and Quantikine® ELISAs for your biomarker validation and subsequent analysis.

Luminex assays are ideal for biomarker discovery when you have samples with limited volume but want to monitor expression of many targets. It allows for simultaneous quantification of up to 50 analytes in just 3.5 hours with as little as 25–50 µL of sample. Luminex multiplex panels give you more data points per sample and are fully customizable so you'll have total control over the targets screened. Once you have potential biomarkers identified, you can validate your results and perform long-term studies with Quantikine ELISAs. Quantikine ELISAs are perfect for both validation and long-term studies when monitoring protein expression level or response to treatment. They are the most published ELISAs on the market and deliver the most accurate and precise results. R&D Systems has over 30 years of expertise developing ELISA assays, ensuring long term assay consistency and reproducibility even between different assay lots.

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MATERIALS AND METHODS

Crohn's patient samples were purchased from BioIVT and apparently healthy patient samples collected in-house. Samples were first analyzed with the R&D Systems Luminex assays (Human XL Cytokine Discovery Panel, Catalog # LKTM014 and Human Luminex Assay, Catalog # LXSAHM) to screen a large panel of targets, some known to have different expression levels according to published literature. A select subset of the identified markers were then validated using in the same samples with either Quantikine Colorimetric Sandwich ELISAs or Quantikine High Sensitivity Colorimetric Sandwich ELISAs when appropriate (Catalog numbers below). Assays were all tested as recommended in the product insert.

RESULTS

We first used the Luminex system to screen circulating levels of 48 cytokines in Crohn's and healthy serum samples. The screen identified multiple cytokines with either similar or elevated serum levels in disease samples compared to apparently healthy samples (Figure 1). Levels of IL-6 and TNF-α were below the limit of quantification (LOQ) in the healthy serum samples, while some of the Crohn's serum samples were detectable. Large differences were observed in IL-8, CRP, and GRO-α with a 21.8-fold, 5.4-fold, and 3.5-fold increase in the Crohn's samples, respectively.

Selected cytokines from the Luminex testing which exhibited increased levels in Crohn's disease samples were further analyzed on Quantikine ELISA kits. The same serum samples were tested in duplicate on either Quantikine or High Sensitivity Quantikine ELISAs (Table 1) for analytes with low circulating levels (Figure 2). All analytes were detected within LOQ for both healthy and diseased serum samples. Average sample coefficient of variance (CV) was below 4% for all samples in all assays. Quantikine results were consistent with Luminex data, demonstrating the ability to easily move from one platform to the other as you progress through the biomarker workflow and your long-term research.

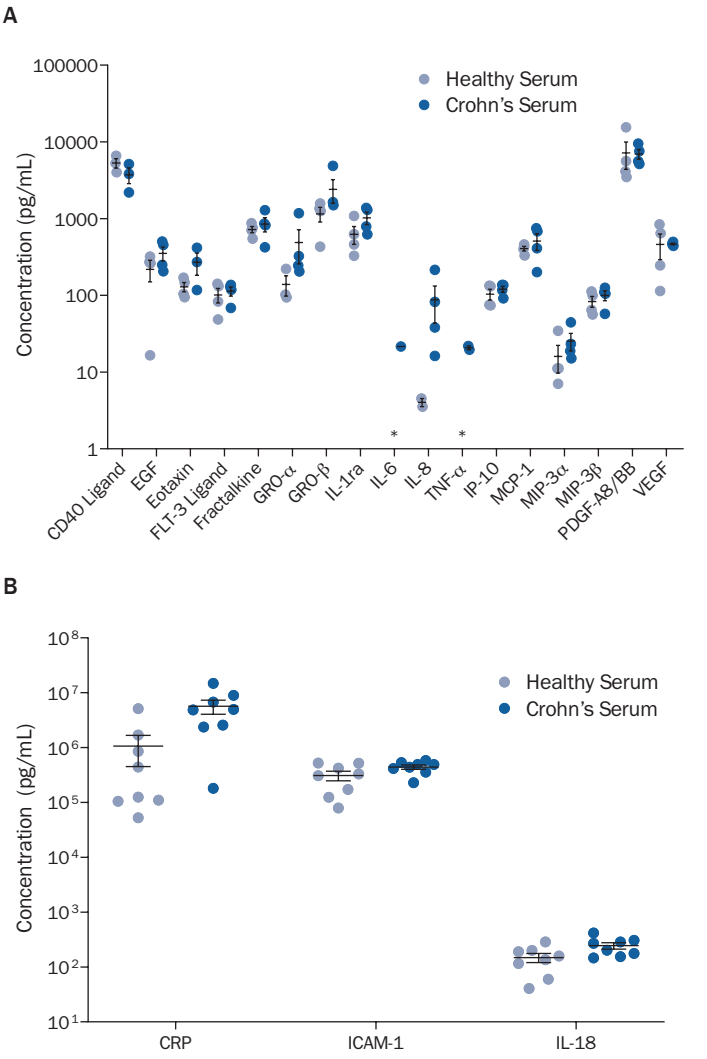

Figure 1. Selected targets from a Luminex assays comparing cytokine levels in serum samples from healthy and Crohn's disease patients. Target concentrations were interpolated using a standard curve and averaged. Serum samples were diluted 1:2 and tested on the Human XL Cytokine Discovery Panel (Catalog # LKTM014) (A). Serum samples were also diluted 1:2 (ICAM-1, IL-18) or 1:200 (CRP) and tested on a Human Luminex Assay (Catalog # LXSAHM) (B). Graph includes the target mean and standard error of the mean (SEM). (*) = target below LOQ.

Target	Kit	Catalog #	Standard Curve Range
CRP	Quantikine® Colorimetric Sandwich ELISA	DCRP00	0.781–50 ng/mL
ICAM-1	Quantikine® Colorimetric Sandwich ELISA	DCIM00	0.625–40 ng/mL
IL-1ra	Quantikine® Colorimetric Sandwich ELISA	DRA00	31.3–2000 pg/mL
IL-18	Quantikine® Colorimetric Sandwich ELISA	DL180	15.6–1000 pg/mL
IL-6	Quantikine HS® Colorimetric Sandwich ELISAs	HS600C	0.156–10 pg/mL
IL-8	Quantikine HS® Colorimetric Sandwich ELISAs	HS800	1.0–64 pg/mL
TNF-α	Quantikine HS® Colorimetric Sandwich ELISAs	HSTA00E	0.156–10 pg/mL

Table 1. Crohn's disease and healthy patient samples run on Quantikine kits to validate Luminex assay data.

CONCLUSIONS

Discovering novel biomarkers for a variety of conditions including Crohn's Disease, cancer, and neurodegenerative disease is crucial to develop new treatments, monitor disease progression, and measure treatment response. Bio-Techne offers a diverse portfolio of immunoassay platforms and assays to optimize your biomarker research workflow which give you fast, reproducible data while conserving your precious samples.

To demonstrate this workflow, we screened over 48 cytokines in healthy vs. Crohn's disease serum samples using the Luminex platform. The data was ready in approximately 3.5 hours. From this data we identified 7 targets to evaluate further with Bio-Techne's legendary Quantikine ELISA product line. The data between the Luminex platform and the Quantikine ELISA's correlated well, demonstrating the ability to move between assay techniques depending on experimental criteria. Whether you are at an initial screening stage or biomarker validation, Bio-Techne has the solution to provide you with accurate and reproducible results.

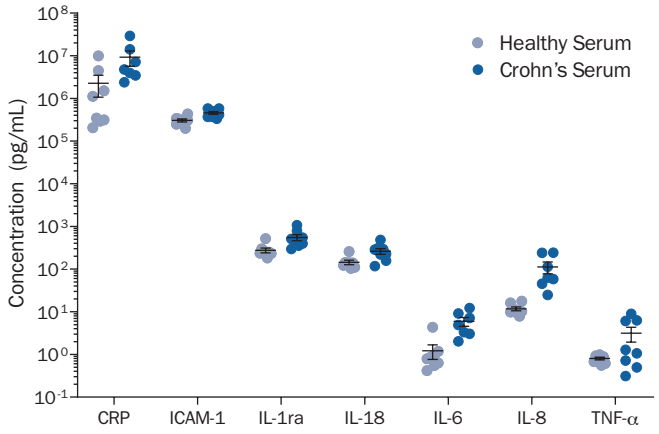

Figure 2. Sample quantification using Quantikine Colorimetric and Quantikine High Sensitivity Colorimetric assays. Graph includes the target mean and standard error of the mean (SEM).