celerion
Translating Science to Medicine

White Paper

Challenges in Biomarker Analysis: SiMoA (Single Molecule Array) Makes The Tiny but Significant Difference

- **Marc-Olivier Montjovent, PhD**, Senior Scientist II, Ligand Binding Services
- **Christopher Acuna**, Analyst, Ligand Binding Services
- **Sebastian Fleire, PhD**, Senior Scientist II, Ligand Binding Services
- **Bettina Bommer, MSc**, Senior Analyst, Ligand Binding Services
- **Michael Gröschl, PhD**, Associate Director - Bioanalytical Laboratory
- **Patrick Brennecke, PhD**, Technical Director Bioanalytical Translational Science
- **Petra Struwe, PhD**, Executive Director Bioanalytical Services

Summary

- Biomarkers are increasingly important in clinical trials as endpoints for patient therapy monitoring. Due to the very low endogenous biomarker concentrations, highly specific and highly sensitive methods are required for their detection and quantification.
- In this study, the performance of four different immunoassay platforms was evaluated: SiMoA SR-X™ (Quanterix®), MESO™ QuickPlex SQ 120 (Mesoscale), Luminex® Bio-Plex® (Bio-Rad) and ELLA™ (Biotechne®).
- Levels of TNF-α and IL-6 cytokines were measured in serum from healthy and chronically diseased individuals using the different technologies.
- Result: SiMoA unequivocally demonstrated to be the most powerful technology within range measurements for all serum samples from healthy as well as chronically ill patients.

1. Chronic Inflammation Biomarkers

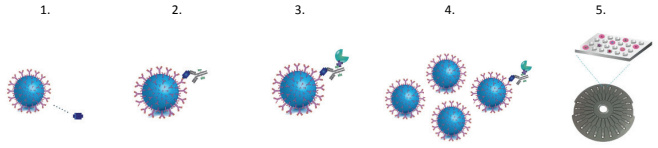
Inflammation is a complex multistep cascade triggered by local tissue injury caused by diverse noxes. Key players in the regulation of inflammatory processes are cytokines and their receptors, ensuring highly specialized immune cells are directed to the site of injury in order to eliminate the noxe. Thereafter inflammation is resolved and cytokine levels are restored to normal levels. Chronic inflammation develops when the dampening of the initial inflammatory response is incomplete or when small amounts of noxes persist. Nowadays chronic inflammation is recognized as one of the key steps prior to the development of very serious diseases like cancer. Therefore it is crucial to be able to monitor tiny changes in cytokine levels of chronically ill patients. In this study we have explored four different technologies in order to monitor low level TNF-α and IL-6 in chronic diseased individuals suffering from psoriasis and compared those to healthy individuals.

- TNF-α has important pro-inflammatory properties, playing crucial roles in the innate and adaptive immunity, cell proliferation, and apoptotic processes. The cytokine is produced by different kinds of cells, including macrophages, monocytes, T-cells, smooth muscle cells, adipocytes, and fibroblasts. Increased concentrations of TNF-α are found in acute e.g. trauma, sepsis, infection and chronic inflammatory conditions like psoriasis (1), for which it could serve as a biomarker for this chronic disease.
- IL-6 (Interleukin-6) is a multifunctional cytokine, originally cloned as an interferon (IFN)-/ antiviral activity from human fibroblasts. In human skin, cutaneous ultraviolet-B irradiation leads to a rapid (12 h) increase in circulating IL-6, suggesting that the skin may be a prominent source of this cytokine. Recently, increased levels of biologically active IL-6 have been reported to be present in blister fluid derived from psoriatic lesions, although bioactivity could not be detected in aqueous extracts of psoriatic scalp. One of the aims of this study was to evaluate if IL-6 could be a biomarker discriminating healthy from psoriatic patients (2).

2. Principle of SiMoA Technology

The SiMoA (single molecule array) technology, by Quanterix, allows the quantification of analyte concentrations at the low pg/mL – fg/mL ranges, using the same reagents as regular ELISA assays (3). In a typical workup, analyte molecules attach to coated beads and get labeled with β-Galactosidase. After distributing the beads to a microarray, with each microwell having the size to capture just one bead, substrate is added, leading to a fluorescent product in those wells containing a labeled bead (Figure 1).

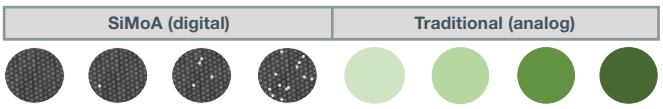
Figure 1. SiMoA Bead-based assay-adapted from (4).



1. Capture of the analyte on paramagnetic coated beads
2. Formation of immuno complex after addition of detection antibody
3. Addition of enzyme conjugate
4. Resuspension in substrate
5. Transfer to arrays and image acquisition

Using laser excitation and CCD camera readout, the concentration is determined by counting, either the ratio of positive to negative microwells (digital, at low concentrations) or the intensity of the overall fluorescence in the microarray (analog, at high concentrations). The combination of analog and digital approaches results in a wide dynamic range and an extraordinary sensitivity in comparison to other immunoassay platforms (Figure 2).

Figure 2. Comparison of digital versus analog signal acquisition-adapted from (5).



Reaction volume ≈ 50 x 10-15 L (2 billion times smaller)

Diffusion defeated = single molecule resolution = ultimate sensitivity

One molecule needed to reach detection limit

Reaction volume = 100 x 10-6 L

Diffusion = dilution = low sensitivity

Millions of molecules needed to reach

3. Material and Methods

A) Platforms/technologies tested

The platforms and kits considered in this study are listed in Table 1.

Table 1. Platform comparison

Platform Kit	Technology/Read-out Quantitation Range (pg/ml)
SR-X (Quanterix) Advantage human assays kit	Fluorescent magnetic bead based assay IL-6: 0.165 – 120 TNF-α: 0.274 - 200
MESO QuickPlex SQ 120 (Mesoscale) V-plex plus pro-inflammatory panel 1 (human) kit	Electrochemiluminescence based assay IL-6: 0.366 – 1496 TNF-α: 0.158 - 646
Luminex Bio-Plex (Bio Rad) Human HS Cytokine premixed kit A	Dual-laser flow-based detection instrument IL-6: 1.66 – 6800 TNF-α: 1.51 - 6200
ELLA (Biotechne) Simple Plex assay cartridge	Microfluidic channel system IL-6: 1.4 - 5304

Adjusted ranges for sample dilution are presented

B) Sample handling

Commercially available sera from healthy as well as diseased donors suffering from psoriasis were analyzed. For each group, 15 donors were included. Matrices were aliquoted and kept at -80°C before analysis. Except for ELLA, standards were analyzed in duplicates and samples and control sera were analyzed in singlets.

B) Statistical analysis

Data were tested for normality (Shapiro Francia test). Non normal data sets were log transformed. The statistic evaluation was performed using either Student t-test or Mann-Whitney-U test.

4. Results for TNF-α

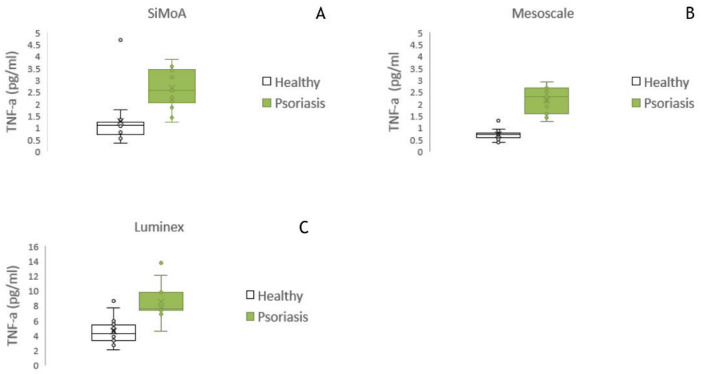
The number of TNF-α measurements that were in quantitation range are shown in Table 2. TNF-α was not measured with the ELLA platform.

Table 2. Samples measured in range for TNF-α using either SiMoA, Mesoscale or Luminex

	SiMoA	Mesoscale	Luminex
Healthy	15/15	15/15	14/15
Psoriasis	15/15	13/15	13/15

Moreover and most importantly TNF-α levels were significantly higher in patients suffering from psoriasis compared to healthy individuals ($p < 0.001$). This observation was made with SiMoA, Mesoscale and Luminex (Figures 3A-3C), demonstrating that TNF-α indeed is an important biomarker for this chronic disease.

Figure 3. TNF-α levels measured in Human sera with SiMoA (A), Mesoscale (B) and Luminex (C) platforms. Box plots with first and third quartiles are depicted. TNF-α levels were significantly higher in patients suffering from psoriasis compared to healthy individuals ($p < 0.001$).



5. Results for IL-6

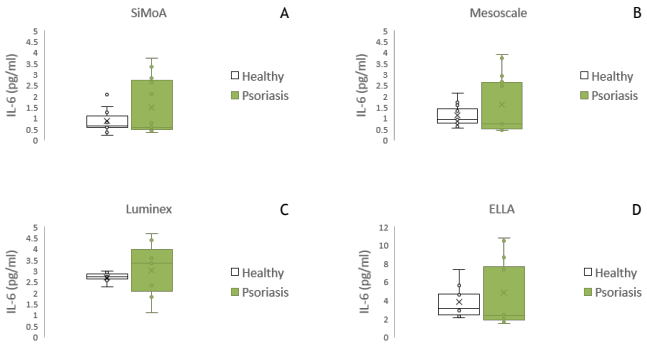
The number of IL-6 measurements that were in quantitation range are shown in Table 3.

Table 3. Samples measured in range for IL-6, using either SiMoA, Mesoscale, Luminex or ELLA

	SiMoA	Mesoscale	Luminex	ELLA
Healthy	15/15	13/15	6/15	11/14
Psoriasis	15/15	14/15	7/15	12/14

However all values of IL-6 were not significantly different when comparing patients from the psoriasis group to the healthy individuals. This observation was made with SiMoA, Mesoscale, Luminex and ELLA (Figures 4A-4D).

Figure 4. IL-6 levels measured in Human sera with SiMoA (A), Mesoscale (B), Luminex (C) and ELLA (D) platforms. Box plots with first and third quartiles are depicted.



6. Discussion and Conclusions

In the present study we have investigated the relevance of low level expression of TNF-α and IL-6 in healthy individuals compared to chronically ill individuals suffering from psoriasis. We examined which of the technologies SiMoA, Mesoscale, Luminex and ELLA is appropriate in order to appreciate, low but significant changes between these two study populations. We have unequivocally demonstrated that the SiMoA technology was able to detect low TNF-α and IL-6 levels in all individuals, in contrast to the other technologies where several individuals were below the quantification range (BLQ). Furthermore, we have shown that TNF-α has a prominent role and can indeed serve as a biomarker for psoriasis, which is not the case for IL-6. Here again SiMoA showed the clearest results.

In conclusion we propose to use SiMoA for chronic disease biomarkers where low level changes between diseased and healthy populations needs to be appreciated, since it was the only technology that allowed for measurement of all tested samples (no below limit of quantitation values). Moreover, this new top edge technology holds promises for patient stratification with appropriate estimations of significant low level changes as shown by TNF-α and IL-6.

References

1. Tsoi LC et al; Identification of fifteen new psoriasis susceptibility loci highlights the role of innate immunity. Nat Genet. 2012 Dec;44(12):1341-8
2. Elder JT et al; Interleukin-6 in psoriasis: expression and mitogenicity studies. 1992;284(6):324-32
3. Rissin DM et al, Single-Molecule enzyme-linked immunosorbent assay detects serum proteins at subfemtomolar concentrations. Nat Biotechnol. 2010 Jun; 28(6): 595–599
4. SIMOA Bead Technology, Quanterix website
5. SIMOA, Scientific Principle of SimoaTM (Single Molecule Array) Technology. Whitepaper 1.0. Quanterix Corp. 2013: 1-2