Global Central Laboratory

Cytokine Assays For Central Lab Testing : A New Frontier

By Els Decoster, PhD, Head of Molecular Biology at BARC Europe

Background

Cytokines as immune system modulators play a major role in the understanding of virtually all pathological processes including cancer, inflammation, infectious diseases, Alzheimer's, autoimmune diseases, allergy and endocrine diseases.

Nowadays, the term 'cytokine' encompasses interferons, interleukins, the chemokine family, mesenchymal growth factors, the tumor necrosis factor family and adipokines.

Over the past 25 years, cytokines have become an important cornerstone of medicine as a diagnostic, prognostic and therapeutic agent in human disease. Cytokines can be divided in functional classes, as shown in Table 1. Testing of all of the mentioned cytokines is currently available at BARC.

Next to cytokines, the determination of soluble receptors may also provide useful information regarding disease states. For example, the levels of soluble TNF receptors are correlated with the clinical stage and progression of both HIV and sepsis. The determination of soluble TNF receptors may also help monitoring cancer and autoimmune diseases. The soluble IL-2 receptor may play a role of prognostic marker for acute lymphoid leukemia as well as for lung cancer.

Cytokines assayed in clinical trials: current and future needs

Recent advances in oncology, through the identification of monoclonal antibodies selectively blocking components of the immune system, including cytokine pathways, made cytokine assays in clinical trials paramount. Yet, endogenous levels of cytokines in humans are so low that conventional methods often fail to provide reliable and reproducible results for cytokine assays. In addition, cytokines act in network, and measurement of several cytokines at once is usually required for achieving clinical relevance. One single method is not available for combining both the requirements of extreme sensitivity and multiplexing and therefore a combination of several techniques matching precisely the clinical and technical requirements of each individual trial is needed. Considering the complexity of those tests, a high level of expertise in both the involved techniques and the interpretation of results is key. Furthermore, study design issues such as timing and manner in which samples are collected and processed from study subjects need to also be carefully considered.

Table 1: examples of cytokines classified by functional class (after Dinarello CA, Eur. J. Immunol. 2007)

Functional Class	Primary Property	Other Effects	Examples
Lymphocyte growth factors	clonal expansion	Th1/Th2/Th17 polarization	IL-17
Th1 cytokine	↑ Th1 response	clonal expansion of CTL	IFN-γ,IL-12,IL-18
Th2 cytokine	↑Th2 response	↑ antibody production	IL-18
Th17 cytokine	↑ Th17 response	autoimmune responses	IL-17, IFNγ
Pro-inflammatory cytokine	↑ inflammatory mediators	↑ innate immune responses	IL-1α, IL-1β, TNF-α, IL-12, IL-18
Anti-inflammatory cytokine	↓ inflammatory genes	↓ cytokine-mediated lethality	IL-10,TGFβ, IL1-RA
Adipokines	pro-inflammatory	↓ autoimmune disease pro-atherogenic	leptin, adiponectin
Gp130 signaling cytokines	growth factors	B-cell activation, acute phase	IL-6
Nerve growth factors	↑ nerve/Schwann cells	B-cell activation	BDNF
Colony stimulating factors	hematopoiesis	pro and anti-inflammatory	GM-CSF
Angiogenic cytokines	neovascularization	pro-metastatic	VEGF,IL-1,IL-6,IL-8
Mesenchymal growth factors	neovascularization	pro-metastatic	FGF,HGF,TGFβ
Other chemokines	↑ cellular migration	↑ cell activation	MCP-1

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Techniques available for cytokine assays

Several techniques are available for cytokine assays, with different characteristics in terms of sensitivity, reproducibility and multiplexing capabilities. Therefore, those techniques are complementary and all must be available for the experts to use them in combination, in order to optimally match the requirements of the study and provide robust results with clinical added value.

1. ELISA

ELISA (Enzyme-linked immunosorbent assay) has been used for decades for the analysis of cytokines. For many years, BARC performs sandwich ELISA for the detection of cytokines. An antibody captured at the bottom of a plate-well provides both antigen capture and immune specificity, while another antibody linked to an enzyme provides detection. The absorbance is measured with a Powerwave micro titer plate reader from BIOTEK, using Gen 5 software for data acquisition.

2. Luminex

Luminex allows for multiple cytokines to be measured at the same time in the same sample.

Principle

The Luminex system, Bio-plex 200, makes use of the xMAP technology to allow the multiplexing of up to theoretically 100 different assays within a single sample. The system uses a liquid suspension array of 100 sets of 5.5 µm beads, each internally dyed with different ratio of two spectrally distinct fluorophores which gives each bead a unique spectral address (Figure 1A). Each set of beads can be conjugated with an analyte-specific capture molecule (Figure 1B). In our multiplex assay, conjugated beads coated with capture antibodies against targets of interest are incubated with the sample in a microplate well and will react specifically with the targets present in the sample. After incubation, any unbound component is removed by a wash step. Detection of the captured targets is made possible by the addition of a biotinylated detection antibody and of a fluorescent-labeled reporter molecule, streptavidin (SE) conjugated with phycoerythrin (PE). Following this last incubation step, the content of each microplate well is drawn into the Bio-plex 200 system and precision fluidics align the beads in a single line that passes through a flow cell where two lasers excite each individual bead (Figure 1C). The red laser excites the dyes in each bead, identifying the spectral address. The green, reporter, laser excites the PE reporter molecule associated with the bead, which allows quantification of the captured analyte. The Bio-plex secure manager software records the fluorescent signals simultaneously for each bead, translating the signals into data, resulting in the quantification of each analyte.

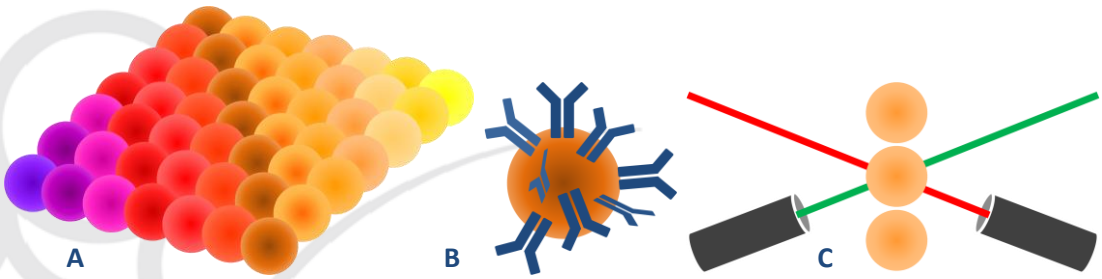


Figure 1 : Principle of the Luminex technology

In 2006, we evaluated the multiplex detection of 4 cytokines : IL-1 beta, IL-6, IL-8 and TNF-alpha in a Rheumatoid Arthritis (RA) trial using the R&D Systems assays with Luminex technology. During validation of these cytokines measurements, we were unable to detect IL-1 beta and TNF-alpha in both healthy volunteers and RA patients, meaning that no discrimination could be made between these two populations with this method. For IL-6 and IL-8 we found no detectable levels in healthy volunteers but elevated levels in the tested RA patients, and this technology was used for many years in our lab for detecting IL-6 and IL-8. For the detection of IL-1 beta and TNF-alpha though, we were forced to use the Ultra Sensitive kits of R&D Systems that used ELISA technology.

In 2013, seeking to offer the latest technological developments to our customers and patients for these key parameters, we cross-validated the Luminex vs Mesoscale techniques for assaying IL-1ß, IL-6, IL-10 and TNF-α (see below).

3. Mesoscale

Further exploring how to develop our capabilities for cytokine analysis, we evaluated in 2012 the Human Pro-Inflammatory 7-Plex assay which detects IL-1ß, IL-6, IL-8, IL-10, IL-12p70, IFN-γ and TNF-α in human serum samples using the mesoscale technology of MSD, which allows multiplexing up to 10 parameters in one well using low sample volume. The assay is available in single-plex and multiplex format.

Principle

A MULTI-SPOT® 96-well plate is used that has been pre-coated on spatially distinct spots with capture antibodies for IL-1ß, IL-6, IL-8, IL-10, IL-12p70, IFN-γ and TNF-α. In a first incubation, analytes in the sample, standard or control bind to the capture antibodies immobilized on the working electrode surface (see Figure 2). After incubation, any unbound component is removed by a wash step. Detection of the captured analytes is established by addition of a SULFO-TAG™ labeled detection antibody. After incubation, any unbound component is removed by a wash step. The read buffer, which provides the appropriate chemical environment for electrochemi-luminescence, is then added to the wells and the plate is loaded into the Sector® Imager for lysis. In the instrument a voltage is applied to the plate electrodes on the bottom of each well which causes the labels bound to the electrode surface to emit light. The instrument measures the intensity of the emitted light to allow quantitative measurement of the analytes, such as in our example IL-1ß, IL-6, IL-8, IL-10, IL-12p70, IFN-γ and TNF-α.

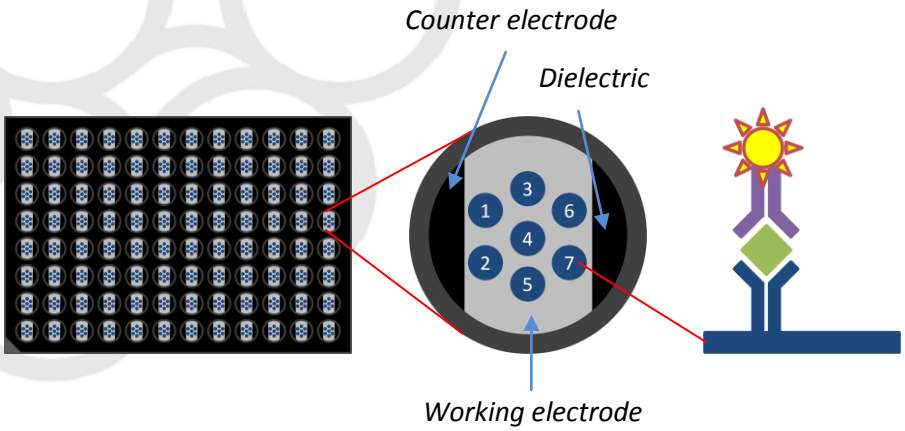


Figure 2 : Principle of the Mesoscale technology

Results

	IL-1 beta	IL-6	IL-8	IL-10	IL12p70	IFN-γ	TNF-α
LOQ (pg/mL)	2.44	0.61	0.61	2.44	2.44	2.44	2.44
Detected healthy volunteers #:	0/33	20/33	33/33	9/33	9/33	6/33	33/33
As percentage:	0%	61%	100%	27%	27%	18%	100%
Reference range	< 2.44	< 2.16	< 19.0	< 3.90	< 3.30	< 2.44	< 10.20
Precision (% CV):							
Intra-assay	*NA	6.3	4.7	13.5	21.1	*NA	7.2
Inter-assay	*NA	16.6	12.4	*NA	*NA	*NA	15.2

*not enough data

Matrix comparison : EDTA plasma versus Serum

To address the question of the variation of the levels of IL-1beta, IL-6, IL-8, IL-10, IL12, IFNgamma and TNF-apha in serum versus EDTA plasma, 24 serum and EDTA plasma samples of the same, apparently healthy, volunteers have been tested using Mesoscale. A summary of the obtained data is shown in the table below. In our hands, IL-6, IL-10 and IFNgamma levels are comparable in serum and in EDTA plasma. For IL-8 and TNF-alpha, higher levels are found in serum than in EDTA plasma.

	Level comparison between serum and EDTA plasma	**Serum / EDTA plasma**
IL-1 beta	None detected in either serum nor EDTA plasma	N/A
IL-6	comparable levels	98.4 % ± 6.3 % (on 7 samples)
IL-8	Serum > EDTA plasma	29.9 % ± 12.2 % (on 24 samples)
IL-10	comparable levels	102.3 % ± 5.2 % (on 6 samples)
IL12	None detected in either serum nor EDTA plasma	N/A
IFNgamma	comparable levels	100.4 % ± 1.6 % (on 2 samples)
TNF-alpha	Serum > EDTA plasma	78.4 % ± 8.2 % (on 23 samples)

Cross-validation Mesoscale vs Luminex

In this cross-validation, 35 samples were assayed using both techniques (Mesoscale and Luminex Biorad assay) for the detection of IL-1ß, IL-6, IL-10 and TNF-α, in order to investigate their respective sensitivity characteristics. The results are presented below and demonstrate that under our conditions IL-6, IL-10 and TNF-α could be measured in more samples using the Mesoscale method than the Luminex one, while IL-1ß remained undetectable in both cases.

	IL-1ß		**IL-6**		**IL-10**		**TNF-α**	
Technique	**LUM**	**MSD**	**LUM**	**MSD**	**LUM**	**MSD**	**LUM**	**MSD**
LOQ (pg/mL)	1.01	2.44	6.35	0.61	11.76	2.44	5.34	2.44
Detected samples #:	0/35	0/35	7/35	32/35	15/35	32/35	10/35	33/35
As percentage:	0 %	0 %	20 %	91 %	43 %	91 %	29%	94 %

Conclusion

For the tested cytokines and in our hands, Mesoscale demonstrates that it is a more sensitive method than Luminex, though it still does not enable us to measure IL-1ß in human samples. As a consequence, a more sensitive method is required for assaying some cytokines, including IL-1ß.

4. Singulex

This method is based on the combination of a microparticle (MP)-based immunoassay and a Single Molecule Counting (SMC) technology, leading to a 50 – 1000 fold higher sensitivity than the other existing immunoassay technologies.

Principle

The Erenna immunoassay is a quantitative fluorescent sandwich immunoassay technique where a capture antibody that is specific for the target to be studied has been pre-coated onto paramagnetic microparticles (see Figure 3). The standards, controls and samples are pipetted into an uncoated microplate well. During incubation, the molecule of interest that is present in the sample binds to the capture antibody on the coated micro particles. Unbound molecules are washed away during the wash step. A Fluor-labeled detection antibody is added to each well and incubated. This detection antibody recognizes and binds to the molecule of interest that has been captured on the micro particles. During the wash step that follows, the micro particles are transferred to a clean plate. Elution buffer is then added and incubated. The elution buffer dissociates the bound proteins from the micro particle surface, releasing the labeled antibodies. These antibodies are separated during transfer to the final micro plate. The plate is loaded on the Erenna System where the labeled molecules are detected and counted. The number of Fluor-labeled detection antibodies counted is directly proportional to the amount of the molecule of interest that was present in the sample. The data acquisition and data processing is performed with the Sgx Link™ software of Singulex.

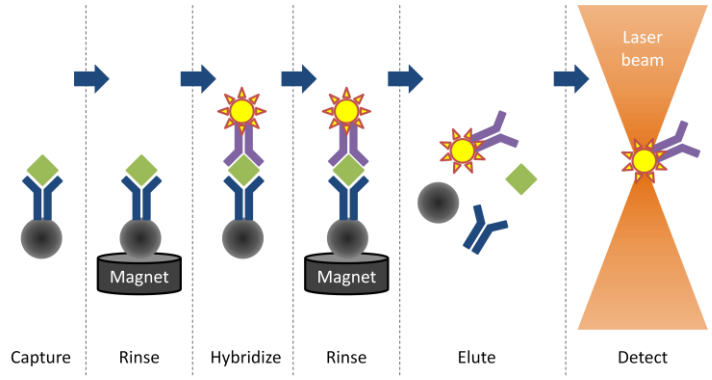


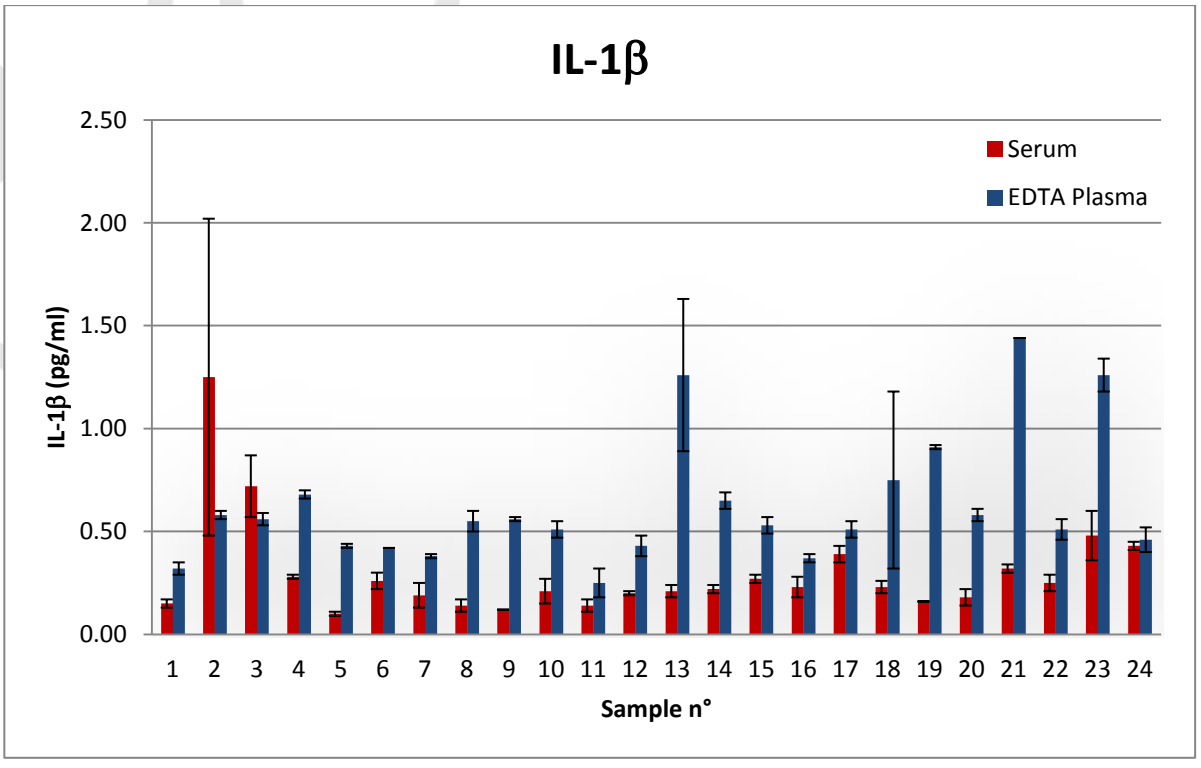
Figure 3 : Principle of the Singulex technology

Validation of the Singulex assay on a selected panel of cytokines

IL-1 beta results

Matrix: Comparison Serum versus EDTA plasma

24 serum samples and 24 EDTA plasma samples of the same healthy volunteers were tested for the presence of IL-1 beta. In all tested samples, detectable IL-1 beta levels were found. From the samples with an acceptable CV, 93% of the samples showed higher levels of IL-1 beta in EDTA plasma than in serum. Consequently, EDTA plasma is the preferred sample matrix for the determination of IL-1 beta using the Singulex technology and the assay validation was performed on EDTA plasma samples.



Analytical performance of the assay

Limit of Quantification:	0.10 pg/mL
Measuring Range :	0.10 pg/mL – 50.00 pg/mL
Accuracy :	High IQC : – 3.9% Bias Mid IQC : – 6.7% Bias Low IQC : + 5.9% Bias
Precision:	Based on our samples, an intra-assay variation of 12.2% CV could be established. Based on our samples an inter-assay variation of 20.5% CV could be established.
Reference range :	In all tested EDTA plasma samples, a detectable value above the LOQ of 0.10 pg/mL was found. Based on 23 samples with an acceptable % CV between duplicate measurements, a range from 0.20 – 0.65 pg/mL was established. Since elevated levels of IL-1 beta are correlated with disease, we set our reference range at < 0.66 pg/mL.

Comparison Singulex versus Mesoscale technology

For all tested serum and EDTA plasma samples which gave a detectable IL-1 beta level with Singulex, a value below LOQ of 2.44 pg/mL for IL-1 beta was found in the Mesoscale assay. In conclusion, Singulex is a much more sensitive method than Mesoscale.

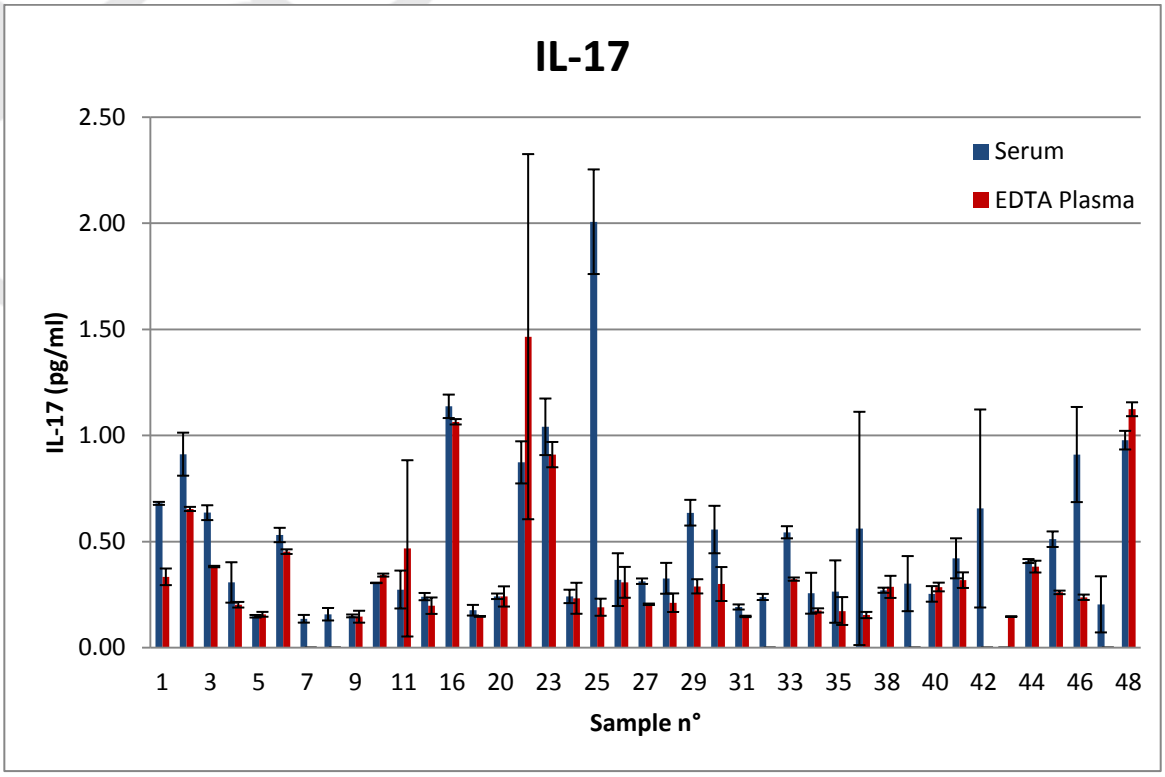
Next to IL-1 beta, we also validated the IL-17 and IL-1 alpha kits of Singulex, since the standard ELISA methods that we are currently using cannot detect these cytokines in samples of apparently healthy volunteers and of clinical trial patients.

IL-17 results

Comparison EDTA plasma versus Serum

The comparison between the IL-17 levels obtained in EDTA plasma and serum of apparently healthy volunteers showed that 19% of serum samples and 10% of EDTA plasma samples showed unacceptable %CV between duplicate measurements. Next, for 19% of the serum samples and for 31% of the EDTA plasma samples no quantifiable level of IL-17 was found (below LOQ of 0.10 pg/mL). For the samples with quantifiable results, a lower level of IL-17 was found in EDTA plasma compared to serum for 50% of them.

Although lower levels of IL-17 are found in EDTA plasma, in our hands EDTA plasma is the matrix which should be used for the detection of IL-17 using Singulex technology since unacceptable high imprecision for the analysis of IL-17 in serum is observed.



Analytical performance of the assay

Limit of Quantification :	0.15 pg/mL
Measuring Range :	0.15 pg/mL – 75.00 pg/mL
Accuracy :	High IQC : – 8.5% Bias Mid IQC : – 7.6% Bias Low IQC : - 4.7% Bias
Precision :	**EDTA plasma** Based on our samples, an intra-assay variation of 9.4% CV could be established. Based on our samples an inter-assay variation of 20.2% CV could be established. **Serum** Based on our samples, an intra-assay variation of 21.0% CV was found. Based on our samples an inter-assay variation of 43.0% CV was found.
Reference range :	**EDTA plasma** Based on 44 out of 48 tested EDTA plasma samples, with an acceptable % CV between duplicate measurements and after exclusion of 3 outliers based on box-plot analysis, based on the results of 41 samples a range from < 0.15 – 0.65 pg/mL was established. Since elevated levels of IL-17 are correlated with disease, we set our reference range at < 0.66 pg/mL. **Serum** Based on 38 out of 48 samples with an acceptable % CV between duplicate measurements, a range from < 0.15 – 1.14 pg/mL was established. Since elevated levels of IL-17 are correlated with disease, we set our reference range at < 1.15 pg/mL.

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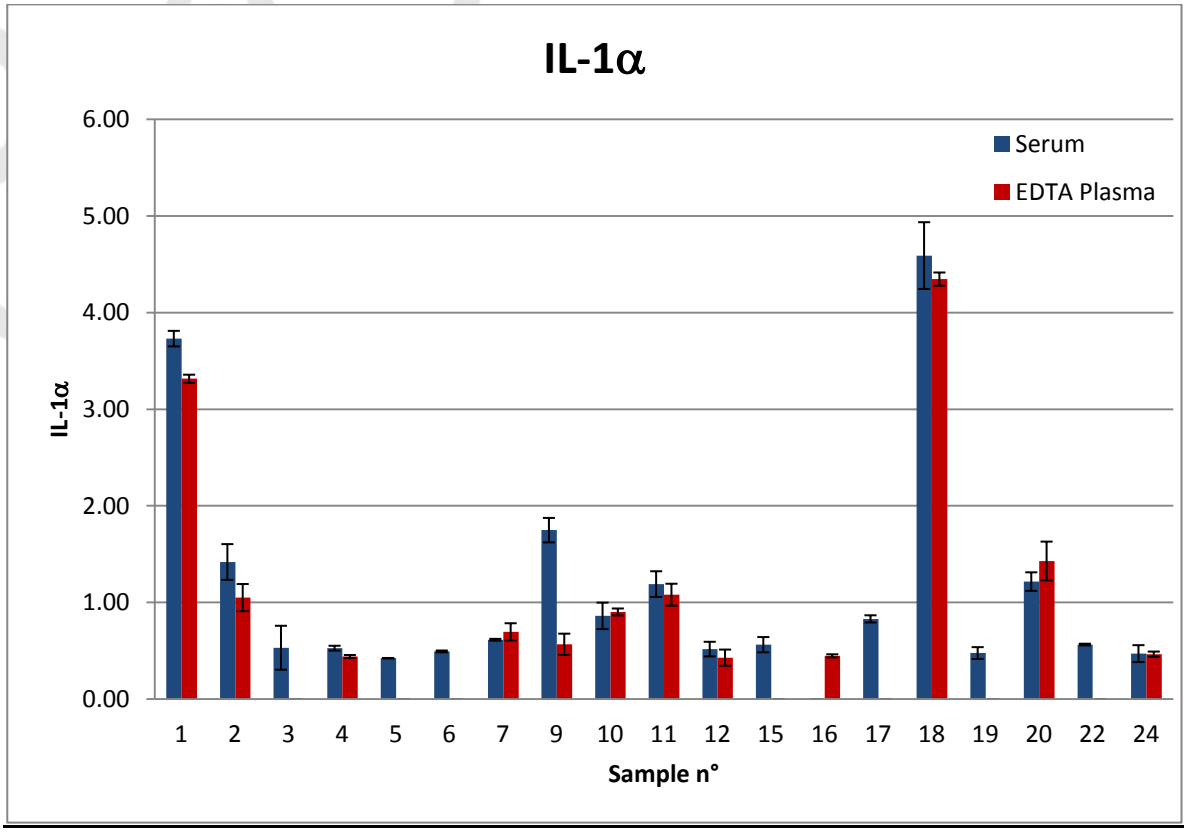
IL-1 alpha results

Comparison EDTA plasma versus Serum

The comparison between the IL-1 alpha levels obtained in EDTA plasma and serum of apparently healthy volunteers showed that 4% of serum samples and none of the EDTA plasma samples showed unacceptable %CV between duplicate measurements. Next, for 21% of the serum samples and for 50% of the EDTA plasma samples no quantifiable level of IL-1 alpha is found (below LOQ of 0.39 pg/mL). For the samples with quantifiable results, for 64% of the samples a lower level of IL-1 alpha is found in EDTA plasma compared to serum. In conclusion, based on concentration levels, we would recommend using serum for the detection of IL-1 alpha using Singulex technology.



Analytical performance of the assay

Limit of Quantification :	0.39 pg/mL
Measuring Range :	0.39 pg/mL – 100.00 pg/mL
Accuracy :	High IQC : +5.3% Bias Mid IQC : + 3.2 % Bias Low IQC : - 1.0% Bias
Precision :	**EDTA plasma** Based on our samples, an intra-assay variation of 8.2% CV could be established. Based on our samples an inter-assay variation of 11.0% CV could be established. **Serum** Based on our samples, an intra-assay variation of 11.3% CV was found. Based on our samples an inter-assay variation of 5.3% CV was found.
Reference range :	**EDTA plasma** Based on 24 tested EDTA plasma samples, with an acceptable % CV between duplicate measurements and after exclusion of 2 outliers based on box-plot analysis, based on the results of 22 samples a range from < 0.39 – 1.43 pg/mL was established. Since elevated levels of IL-1 alpha are correlated with disease, we set our reference range at < 1.44 pg/mL. **Serum** Based on 22 out of 24 samples with an acceptable % CV between duplicate measurements, and after exclusion of 2 outliers based on box-plot analysis, based on the results of 20 samples a range from < 0.39 – 1.75 pg/mL was established. Since elevated levels of IL-1 alpha are correlated with disease, we set our reference range at < 1.76 pg/mL

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Fast-moving fields: the importance of flexible R&D capabilities in central lab

At BARC, we are continuously looking for meaningful innovative technologies. The implementation of the Singulex technology is a result of our quest to find a way that enables us to measure key parameters that could not be detected with current standard immuno-assay techniques. The implementation of the technology proved challenging, as a lot of method optimization during validation was necessary to meet our clinical trial-level quality standards for immuno-assays. At this moment, we have successfully validated IL-1beta, IL-17 and IL-1 alpha on Singulex, and are further extending the range of parameters we can detect with this method.

Next to Singulex, and in order to broaden our cytokine portfolio and to offer complete flexibility in studying different cytokines and their interactions, we are also currently validating 40 selected parameters using the new V-plex plus kit from Mesoscale.

At the heart of cytokine assays for clinical trials: expertise

As we have seen, assaying cytokines requires several specific skills:

- Extensive testing capabilities that allow for freely choosing the technology that optimally fits one's strategy
- Strong capabilities in the design of the testing procedure, the implementation of relevant controls and the method validation
- Capabilities of proposing additional tests with added value.

Therefore, disposing of the most sensitive tools for cytokine assay is valuable only if it is supported by an extensive expertise in the field. This notion is of special importance when it comes to cytokines, since they are involved in virtually all therapeutic areas, thus requiring specific knowledge for each type of clinical trial.

A dedicated R&D team, headed by a scientist with more than 10 years of experience in clinical trial field, enables BARC to fulfill this mission and to implement new assays in a smooth, efficient and meaningful way.

Conclusion

The growing interest for cytokine assays in clinical trials reflects the increasing awareness of their relevance in many therapeutic areas and pathological pathways. At the same time, new techniques are developed that enable us to detect parameters which are present at very low concentration levels in bodily fluids. These new assays by definition need to have extremely low LoQ levels. Since they are not only very sensitive but also susceptible to variations, they must be carried out by highly experienced lab professionals. Finally, it is crucial to determine the optimal strategy for delivering results with added value which are in line with the specifics of the clinical trial and the therapeutic effects to be investigated.

For more information, please contact edecoster@barclab.com and visit www.barclab.com.

Appendices

IL-1β with Mesoscale

IL-1β (pg/ml)	EDTA plasma					IL-1β (pg/ml)	serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	< 2.44	< 2.44	< 2.44	/	/	1	< 2.44	< 2.44	< 2.44	/	/
2	< 2.44	< 2.44	< 2.44	/	/	2	< 2.44	< 2.44	< 2.44	/	/
3	< 2.44	< 2.44	< 2.44	/	/	3	< 2.44	< 2.44	< 2.44	/	/
4	< 2.44	< 2.44	< 2.44	/	/	4	< 2.44	< 2.44	< 2.44	/	/
5	< 2.44	< 2.44	< 2.44	/	/	5	< 2.44	< 2.44	< 2.44	/	/
6	< 2.44	< 2.44	< 2.44	/	/	6	< 2.44	< 2.44	< 2.44	/	/
7	< 2.44	< 2.44	< 2.44	/	/	7	< 2.44	< 2.44	< 2.44	/	/
8	< 2.44	< 2.44	< 2.44	/	/	8	< 2.44	< 2.44	< 2.44	/	/
9	< 2.44	< 2.44	< 2.44	/	/	9	< 2.44	< 2.44	< 2.44	/	/
10	< 2.44	< 2.44	< 2.44	/	/	10	< 2.44	< 2.44	< 2.44	/	/
11	< 2.44	< 2.44	< 2.44	/	/	11	< 2.44	< 2.44	< 2.44	/	/
12	< 2.44	< 2.44	< 2.44	/	/	12	< 2.44	< 2.44	< 2.44	/	/
13	< 2.44	< 2.44	< 2.44	/	/	13	< 2.44	< 2.44	< 2.44	/	/
14	< 2.44	< 2.44	< 2.44	/	/	14	< 2.44	< 2.44	< 2.44	/	/
15	< 2.44	< 2.44	< 2.44	/	/	15	< 2.44	< 2.44	< 2.44	/	/
16	< 2.44	< 2.44	< 2.44	/	/	16	< 2.44	< 2.44	< 2.44	/	/
17	< 2.44	< 2.44	< 2.44	/	/	17	< 2.44	< 2.44	< 2.44	/	/
18	< 2.44	< 2.44	< 2.44	/	/	18	< 2.44	< 2.44	< 2.44	/	/
19	< 2.44	< 2.44	< 2.44	/	/	19	< 2.44	< 2.44	< 2.44	/	/
20	< 2.44	< 2.44	< 2.44	/	/	20	< 2.44	< 2.44	< 2.44	/	/
21	< 2.44	< 2.44	< 2.44	/	/	21	< 2.44	< 2.44	< 2.44	/	/
22	< 2.44	< 2.44	< 2.44	/	/	22	< 2.44	< 2.44	< 2.44	/	/
23	< 2.44	< 2.44	< 2.44	/	/	23	< 2.44	< 2.44	< 2.44	/	/
24	< 2.44	< 2.44	< 2.44	/	/	24	< 2.44	< 2.44	< 2.44	/	/

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IL-6 with Mesoscale

IL-6 (pg/ml)	EDTA plasma					IL-6 (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	< 0.61	< 0.61	< 0.61	/	/	1	< 0.61	< 0.61	< 0.61	/	/
2	< 0.61	< 0.61	< 0.61	/	/	2	< 0.61	< 0.61	< 0.61	/	/
3	< 0.61	< 0.61	< 0.61	/	/	3	< 0.61	< 0.61	< 0.61	/	/
4	2.01	1.99	2.00	0.013	0.7	4	2.01	1.90	1.95	0.073	3.8
5	< 0.61	0.64	< 0.61	/	/	5	< 0.61	< 0.61	< 0.61	/	/
6	0.78	0.75	0.76	0.021	2.7	6	0.88	0.91	0.89	0.018	2.0
7	0.63	< 0.61	< 0.61	/	/	7	< 0.61	< 0.61	< 0.61	/	/
8	1.20	1.27	1.23	0.043	3.5	8	1.14	1.22	1.18	0.059	5.0
9	1.57	1.48	1.52	0.063	4.1	9	1.46	1.61	1.54	0.110	7.1
10	0.98	1.13	1.06	0.102	9.7	10	1.08	1.01	1.04	0.048	4.6
11	0.74	0.79	0.76	0.039	5.1	11	0.81	0.82	0.81	0.007	0.8
12	< 0.61	< 0.61	< 0.61	/	/	12	< 0.61	< 0.61	< 0.61	/	/
13	< 0.61	< 0.61	< 0.61	/	/	13	< 0.61	< 0.61	< 0.61	/	/
14	< 0.61	< 0.61	< 0.61	/	/	14	< 0.61	< 0.61	< 0.61	/	/
15	< 0.61	< 0.61	< 0.61	/	/	15	< 0.61	< 0.61	< 0.61	/	/
16	< 0.61	< 0.61	< 0.61	/	/	16	< 0.61	< 0.61	< 0.61	/	/
17	1.57	1.61	1.59	0.026	1.6	17	1.58	1.52	1.55	0.043	2.8
18	0.63	< 0.61	< 0.61	/	/	18	< 0.61	< 0.61	< 0.61	/	/
19	< 0.61	< 0.61	< 0.61	/	/	19	< 0.61	< 0.61	< 0.61	/	/
20	< 0.61	< 0.61	< 0.61	/	/	20	< 0.61	< 0.61	< 0.61	/	/
21	< 0.61	< 0.61	< 0.61	/	/	21	< 0.61	< 0.61	< 0.61	/	/
22	< 0.61	< 0.61	< 0.61	/	/	22	< 0.61	< 0.61	< 0.61	/	/
23	< 0.61	< 0.61	< 0.61	/	/	23	< 0.61	< 0.61	< 0.61	/	/
24	< 0.61	< 0.61	< 0.61	/	/	24	< 0.61	< 0.61	< 0.61	/	/

BARC
Global Central Laboratory

IL-8 with Mesoscale

IL-8 (pg/ml)	EDTA plasma					IL-8 (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	6.68	6.86	6.77	0.132	2.0	1	9.85	10.30	10.10	0.290	2.9
2	4.16	4.32	4.24	0.113	2.7	2	10.20	10.20	10.20	0.054	0.5
3	2.37	2.16	2.26	0.145	6.4	3	10.40	10.80	10.60	0.279	2.7
4	2.42	2.58	2.50	0.114	4.6	4	12.30	12.50	12.40	0.176	1.4
5	4.80	4.89	4.85	0.065	1.4	5	15.10	14.40	14.70	0.505	3.4
6	1.88	1.86	1.87	0.012	0.7	6	7.78	7.87	7.83	0.059	0.7
7	2.03	1.94	1.99	0.064	3.2	7	6.43	6.09	6.26	0.242	3.9
8	3.48	3.66	3.57	0.127	3.6	8	11.90	12.80	12.40	0.655	5.3
9	4.81	4.77	4.79	0.027	0.6	9	13.20	13.10	13.10	0.066	0.5
10	4.01	4.01	4.01	0.004	0.1	10	14.60	14.10	14.30	0.314	2.2
11	3.68	3.64	3.66	0.025	0.7	11	11.50	11.40	11.40	0.094	0.8
12	4.41	4.44	4.42	0.020	0.5	12	6.83	6.88	6.85	0.041	0.6
13	2.37	2.40	2.38	0.018	0.8	13	10.20	10.70	10.50	0.391	3.7
14	5.13	5.33	5.23	0.143	2.7	14	18.70	18.40	18.50	0.161	0.9
15	1.94	1.92	1.93	0.016	0.8	15	11.60	11.80	11.70	0.122	1.0
16	1.84	1.88	1.86	0.024	1.3	16	9.34	9.31	9.33	0.023	0.2
17	4.08	4.02	4.05	0.043	1.1	17	14.70	14.60	14.60	0.068	0.5
18	2.22	2.24	2.23	0.013	0.6	18	9.12	9.18	9.15	0.040	0.4
19	3.18	3.41	3.30	0.169	5.1	19	11.00	11.30	11.10	0.201	1.8
20	2.96	3.40	3.18	0.312	9.8	20	12.80	12.40	12.60	0.267	2.1
21	4.30	4.39	4.35	0.064	1.5	21	20.80	20.90	20.90	0.112	0.5
22	4.38	4.51	4.44	0.089	2.0	22	20.90	20.30	20.60	0.453	2.2
23	2.89	2.96	2.92	0.049	1.7	23	12.90	13.10	13.00	0.180	1.4
24	1.92	1.82	1.87	0.070	3.7	24	6.63	6.43	6.53	0.142	2.2

IL-10 with Mesoscale

IL-10 (pg/ml)	EDTA plasma					IL-10 (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	2.52	2.53	2.52	0.006	0.2	1	< 2.44	2.55	< 2.44	/	/
2	2.45	< 2.44	< 2.44	/	/	2	2.62	2.48	2.55	0.097	3.8
3	< 2.44	< 2.44	< 2.44	/	/	3	< 2.44	< 2.44	< 2.44	/	/
4	2.82	2.8	2.81	0.012	0.4	4	2.82	2.6	2.71	0.152	5.6
5	< 2.44	< 2.44	< 2.44	/	/	5	< 2.44	< 2.44	< 2.44	/	/
6	< 2.44	< 2.44	< 2.44	/	/	6	< 2.44	< 2.44	< 2.44	/	/
7	< 2.44	< 2.44	< 2.44	/	/	7	< 2.44	< 2.44	< 2.44	/	/
8	3.28	2.67	2.98	0.429	14.4	8	2.84	2.98	2.91	0.097	3.3
9	< 2.44	< 2.44	< 2.44	/	/	9	< 2.44	< 2.44	< 2.44	/	/
10	2.82	3.13	2.98	0.224	7.5	10	3.26	2.87	3.07	0.278	9.1
11	< 2.44	< 2.44	< 2.44	/	/	11	< 2.44	< 2.44	< 2.44	/	/
12	2.47	2.69	2.58	0.152	5.9	12	2.58	2.71	2.64	0.091	3.5
13	< 2.44	< 2.44	< 2.44	/	/	13	< 2.44	< 2.44	< 2.44	/	/
14	< 2.44	< 2.44	< 2.44	/	/	14	< 2.44	< 2.44	< 2.44	/	/
15	< 2.44	< 2.44	< 2.44	/	/	15	< 2.44	< 2.44	< 2.44	/	/
16	< 2.44	< 2.44	< 2.44	/	/	16	< 2.44	< 2.44	< 2.44	/	/
17	< 2.44	2.79	2.61	0.255	9.8	17	2.70	2.60	2.65	0.072	2.7
18	< 2.44	< 2.44	< 2.44	/	/	18	< 2.44	< 2.44	< 2.44	/	/
19	< 2.44	< 2.44	< 2.44	/	/	19	< 2.44	< 2.44	< 2.44	/	/
20	2.58	< 2.44	< 2.44	/	/	20	< 2.44	< 2.44	< 2.44	/	/
21	< 2.44	< 2.44	< 2.44	/	/	21	< 2.44	< 2.44	< 2.44	/	/
22	< 2.44	< 2.44	< 2.44	/	/	22	< 2.44	< 2.44	< 2.44	/	/
23	3.39	3.15	3.27	0.166	5.1	23	3.77	3.59	3.68	0.124	3.4
24	< 2.44	< 2.44	< 2.44	/	/	24	< 2.44	< 2.44	< 2.44	/	/

IL-12 with Mesoscale

IL-12 (pg/ml)	EDTA plasma					IL-12 (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	< 2.44	< 2.44	< 2.44	/	/	1	< 2.44	< 2.44	< 2.44	/	/
2	< 2.44	< 2.44	< 2.44	/	/	2	< 2.44	< 2.44	< 2.44	/	/
3	< 2.44	< 2.44	< 2.44	/	/	3	< 2.44	< 2.44	< 2.44	/	/
4	< 2.44	< 2.44	< 2.44	/	/	4	< 2.44	< 2.44	< 2.44	/	/
5	< 2.44	< 2.44	< 2.44	/	/	5	< 2.44	< 2.44	< 2.44	/	/
6	< 2.44	< 2.44	< 2.44	/	/	6	< 2.44	< 2.44	< 2.44	/	/
7	< 2.44	< 2.44	< 2.44	/	/	7	< 2.44	< 2.44	< 2.44	/	/
8	< 2.44	< 2.44	< 2.44	/	/	8	< 2.44	< 2.44	< 2.44	/	/
9	< 2.44	< 2.44	< 2.44	/	/	9	< 2.44	< 2.44	< 2.44	/	/
10	< 2.44	< 2.44	< 2.44	/	/	10	< 2.44	< 2.44	< 2.44	/	/
11	< 2.44	< 2.44	< 2.44	/	/	11	< 2.44	< 2.44	< 2.44	/	/
12	< 2.44	< 2.44	< 2.44	/	/	12	< 2.44	< 2.44	< 2.44	/	/
13	< 2.44	< 2.44	< 2.44	/	/	13	< 2.44	< 2.44	< 2.44	/	/
14	< 2.44	< 2.44	< 2.44	/	/	14	< 2.44	< 2.44	< 2.44	/	/
15	< 2.44	< 2.44	< 2.44	/	/	15	< 2.44	< 2.44	< 2.44	/	/
16	< 2.44	< 2.44	< 2.44	/	/	16	< 2.44	< 2.44	< 2.44	/	/
17	< 2.44	< 2.44	< 2.44	/	/	17	< 2.44	< 2.44	< 2.44	/	/
18	< 2.44	< 2.44	< 2.44	/	/	18	< 2.44	< 2.44	< 2.44	/	/
19	< 2.44	< 2.44	< 2.44	/	/	19	< 2.44	< 2.44	< 2.44	/	/
20	< 2.44	< 2.44	< 2.44	/	/	20	< 2.44	< 2.44	< 2.44	/	/
21	< 2.44	< 2.44	< 2.44	/	/	21	< 2.44	< 2.44	< 2.44	/	/
22	< 2.44	< 2.44	< 2.44	/	/	22	< 2.44	< 2.44	< 2.44	/	/
23	< 2.44	< 2.44	< 2.44	/	/	23	< 2.44	< 2.44	< 2.44	/	/
24	< 2.44	< 2.44	< 2.44	/	/	24	< 2.44	< 2.44	< 2.44	/	/

IFNgamma with Mesoscale

IFNγ (pg/ml)	EDTA plasma					IFNγ (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	< 2.44	< 2.44	< 2.44	/	/	1	< 2.44	< 2.44	< 2.44	/	/
2	< 2.44	< 2.44	< 2.44	/	/	2	< 2.44	< 2.44	< 2.44	/	/
3	< 2.44	< 2.44	< 2.44	/	/	3	< 2.44	< 2.44	< 2.44	/	/
4	< 2.44	< 2.44	< 2.44	/	/	4	< 2.44	< 2.44	< 2.44	/	/
5	< 2.44	< 2.44	< 2.44	/	/	5	< 2.44	< 2.44	< 2.44	/	/
6	< 2.44	< 2.44	< 2.44	/	/	6	< 2.44	< 2.44	< 2.44	/	/
7	< 2.44	< 2.44	< 2.44	/	/	7	< 2.44	< 2.44	< 2.44	/	/
8	< 2.44	< 2.44	< 2.44	/	/	8	< 2.44	< 2.44	< 2.44	/	/
9	< 2.44	< 2.44	< 2.44	/	/	9	< 2.44	< 2.44	< 2.44	/	/
10	< 2.44	< 2.44	< 2.44	/	/	10	< 2.44	< 2.44	< 2.44	/	/
11	< 2.44	< 2.44	< 2.44	/	/	11	< 2.44	< 2.44	< 2.44	/	/
12	< 2.44	< 2.44	< 2.44	/	/	12	< 2.44	< 2.44	< 2.44	/	/
13	< 2.44	< 2.44	< 2.44	/	/	13	< 2.44	< 2.44	< 2.44	/	/
14	< 2.44	< 2.44	< 2.44	/	/	14	< 2.44	< 2.44	< 2.44	/	/
15	< 2.44	< 2.44	< 2.44	/	/	15	< 2.44	< 2.44	< 2.44	/	/
16	< 2.44	< 2.44	< 2.44	/	/	16	< 2.44	< 2.44	< 2.44	/	/
17	6.25	5.79	6.02	0.321	5.3	17	5.93	5.86	5.9	0.049	0.8
18	< 2.44	< 2.44	< 2.44	/	/	18	< 2.44	< 2.44	< 2.44	/	/
19	< 2.44	< 2.44	< 2.44	/	/	19	< 2.44	< 2.44	< 2.44	/	/
20	< 2.44	< 2.44	< 2.44	/	/	20	< 2.44	< 2.44	< 2.44	/	/
21	10.10	9.05	9.56	0.732	7.7	21	10.10	9.20	9.67	0.658	6.8
22	< 2.44	< 2.44	< 2.44	/	/	22	< 2.44	< 2.44	< 2.44	/	/
23	< 2.44	< 2.44	< 2.44	/	/	23	< 2.44	< 2.44	< 2.44	/	/
24	< 2.44	< 2.44	< 2.44	/	/	24	< 2.44	< 2.44	< 2.44	/	/

TNFalpha with Mesoscale

TNFα (pg/ml)	EDTA plasma					TNFα (pg/ml)	Serum				
Sample	Det1	Det2	Mean	SD	%CV	Sample	Det1	Det2	Mean	SD	%CV
1	5.46	5.40	5.43	0.040	0.7	1	5.64	6.22	5.93	0.406	6.8
2	5.12	5.13	5.13	0.007	0.1	2	6.92	6.67	6.79	0.179	2.6
3	3.46	3.78	3.62	0.224	6.2	3	6.56	6.34	6.45	0.152	2.4
4	5.57	5.73	5.65	0.113	2.0	4	6.28	6.81	6.55	0.371	5.7
5	4.02	4.07	4.04	0.034	0.8	5	4.93	5.01	4.97	0.054	1.1
6	3.99	4.07	4.03	0.054	1.3	6	5.83	5.81	5.82	0.013	0.2
7	4.52	4.67	4.59	0.108	2.3	7	6.16	6.03	6.10	0.093	1.5
8	6.92	6.53	6.72	0.278	4.1	8	8.34	8.71	8.53	0.262	3.1
9	3.67	3.41	3.54	0.191	5.3	9	4.66	4.34	4.50	0.229	5.1
10	< 2.44	< 2.44	< 2.44	/	/	10	< 2.44	< 2.44	< 2.44	/	/
11	4.11	3.84	3.97	0.190	4.8	11	5.19	5.66	5.43	0.334	6.2
12	4.21	3.95	4.08	0.183	4.5	12	5.43	5.63	5.53	0.140	2.5
13	4.33	4.00	4.16	0.234	5.6	13	4.95	4.90	4.93	0.038	0.8
14	4.10	3.92	4.01	0.129	3.2	14	5.69	5.83	5.76	0.101	1.8
15	2.87	2.86	2.86	0.010	0.3	15	3.47	3.78	3.62	0.215	5.9
16	2.70	2.60	2.65	0.076	2.9	16	3.32	3.15	3.23	0.119	3.7
17	10.70	11.00	10.80	0.198	1.8	17	12.20	12.30	12.30	0.034	0.3
18	5.18	5.31	5.25	0.091	1.7	18	6.82	6.76	6.79	0.043	0.6
19	3.98	4.36	4.17	0.273	6.5	19	4.97	4.57	4.77	0.278	5.8
20	3.57	3.11	3.34	0.329	9.9	20	4.84	4.49	4.67	0.249	5.3
21	6.42	6.19	6.31	0.163	2.6	21	7.01	6.62	6.82	0.279	4.1
22	4.29	4.47	4.38	0.129	3.0	22	5.24	5.20	5.22	0.034	0.6
23	5.12	4.60	4.86	0.364	7.5	23	7.04	6.98	7.01	0.038	0.5
24	3.45	3.69	3.57	0.167	4.7	24	4.50	4.52	4.51	0.014	0.3

Luminex versus Mesoscale

pg/mL	IL-1ß		IL-6		IL-10		TNF-α	
Samples	Luminex	MSD	Luminex	MSD	Luminex	MSD	Luminex	MSD
1	<1.01	<2.44	<6.35	<0.61	<11.76	<2.44	<5.34	2.58
2	<1.01	<2.44	<6.35	2.60	<11.76	<2.44	<5.34	2.57
3	<1.01	<2.44	<6.35	0.91	42.27	7.82	<5.34	3.27
4	<1.01	<2.44	<6.35	1.67	<11.76	<2.44	<5.34	2.59
5	<1.01	<2.44	<6.35	0.83	<11.76	2.96	<5.34	3.20
6	<1.01	<2.44	<6.35	0.76	53.19	12.30	<5.34	3.27
7	<1.01	<2.44	<6.35	<0.61	14.17	<2.44	<5.34	2.49
8	<1.01	<2.44	9.70	6.17	19.48	3.49	<5.34	2.65
9	<1.01	<2.44	<6.35	<0.61	17.57	5.70	<5.34	<2.44
10	<1.01	<2.44	<6.35	1.53	23.48	2.60	<5.34	<2.44
11	<1.01	<2.44	16.66	11.20	<11.76	10.63	7.64	25.33
12	<1.01	<2.44	<6.35	3.26	<11.76	15.37	12.32	31.42
13	<1.01	<2.44	<6.35	2.27	<11.76	9.65	6.42	17.34
14	<1.01	<2.44	<6.35	3.21	<11.76	2.57	<5.34	8.75
15	<1.01	<2.44	<6.35	2.14	<11.76	12.40	<5.34	11.89
16	<1.01	<2.44	<6.35	2.94	115.52	158.93	10.70	37.10
17	<1.01	<2.44	<6.35	2.89	47.11	75.10	<5.34	14.52
18	<1.01	<2.44	<6.35	1.98	<11.76	4.78	<5.34	9.48
19	<1.01	<2.44	<6.35	1.30	<11.76	10.91	<5.34	6.59
20	<1.01	<2.44	<6.35	2.01	92.31	130.67	5.94	16.44
21	<1.01	<2.44	<6.35	3.21	<11.76	18.96	<5.34	15.30
22	<1.01	<2.44	<6.35	1.79	<11.76	24.11	<5.34	12.32
23	<1.01	<2.44	<6.35	1.62	<11.76	13.88	<5.34	11.66
24	<1.01	<2.44	9.70	6.41	<11.76	7.17	<5.34	13.31
25	<1.01	<2.44	<6.35	1.88	<11.76	11.45	<5.34	10.11
26	<1.01	<2.44	<6.35	1.19	<11.76	3.67	<5.34	7.18
27	<1.01	<2.44	<6.35	4.49	<11.76	6.00	<5.34	7.52
28	<1.01	<2.44	<6.35	2.97	132.57	282.18	<5.34	13.1
29	<1.01	<2.44	41.03	23.75	<11.76	12.88	9.27	30.3
30	<1.01	<2.44	6.91	7.03	13.68	45.26	6.62	25.4
31	<1.01	<2.44	7.09	6.52	<11.76	8.89	<5.34	12.4
32	<1.01	<2.44	<6.35	3.86	143.10	262.49	7.71	24.6
33	<1.01	<2.44	<6.35	5.09	248.96	221.44	6.28	23.50
34	<1.01	<2.44	<6.35	2.80	104.79	115.15	<5.34	18.76
35	<1.01	<2.44	7.61	6.69	98.47	150.46	21.37	52.7

IL-1β with Singulex

IL-1β (pg/ml)	Serum					EDTA plasma				
Sample	Det 1	Det 2	Mean	SD	CV (%)	Det 1	Det 2	Mean	SD	CV (%)
1	0.17	0.14	0.15	0.02	14.7	0.34	0.30	0.32	0.03	8.6
2	0.71	1.78	1.25	0.77	61.2	0.59	0.56	0.58	0.02	3.6
3	0.82	0.62	0.72	0.15	20.4	0.59	0.54	0.56	0.03	6.2
4	0.29	0.27	0.28	0.01	4.7	0.67	0.69	0.68	0.02	2.5
5	0.11	0.09	0.10	0.01	11.6	0.43	0.44	0.43	0.01	1.3
6	0.30	0.24	0.26	0.04	15.9	0.42	0.42	0.42	0.00	1.0
7	0.23	0.15	0.19	0.06	30.9	0.39	0.37	0.38	0.01	3.8
8	0.17	0.13	0.14	0.03	18.8	0.59	0.52	0.55	0.05	9.1
9	0.13	0.12	0.12	0.00	2.0	0.56	0.57	0.56	0.01	1.4
10	0.17	0.25	0.21	0.06	28.7	0.54	0.49	0.51	0.04	6.9
11	0.17	0.13	0.14	0.03	19.6	0.21	0.31	0.25	0.07	26.6
12	0.20	0.21	0.20	0.01	3.2	0.40	0.47	0.43	0.05	10.9
13	0.23	0.19	0.21	0.03	14.7	1.00	1.53	1.26	0.37	29.7
14	0.20	0.23	0.22	0.02	10.5	0.68	0.63	0.65	0.04	5.8
15	0.26	0.28	0.27	0.02	7.2	0.50	0.56	0.53	0.04	8.1
16	0.19	0.27	0.23	0.05	22.2	0.36	0.39	0.37	0.02	6.1
17	0.42	0.36	0.39	0.04	10.9	0.54	0.48	0.51	0.04	8.5
18	0.21	0.25	0.23	0.03	11.6	0.45	1.04	0.75	0.43	57.1
19	0.16	0.16	0.16	0.00	0.7	0.90	0.91	0.91	0.01	0.8
20	0.21	0.15	0.18	0.04	24.1	0.56	0.61	0.58	0.03	6.0
21	0.31	0.34	0.32	0.02	5.8	1.44	1.44	1.44	0.00	0.1
22	0.22	0.28	0.25	0.04	15.4	0.55	0.47	0.51	0.05	10.4
23	0.56	0.39	0.48	0.12	25.0	1.20	1.31	1.26	0.08	6.0
24	0.42	0.44	0.43	0.02	3.8	0.42	0.50	0.46	0.06	12.7

IL-17 with Singulex

IL-17 (pg/ml)	Serum					EDTA plasma				
Sample	Det 1	Det 2	Mean	SD	CV (%)	Det 1	Det 2	Mean	SD	CV (%)
1	0.68	0.68	0.68	0.01	1.0	0.31	0.36	0.33	0.04	11.7
2	0.98	0.84	0.91	0.10	11.1	0.66	0.65	0.65	0.01	1.5
3	0.66	0.61	0.64	0.03	5.5	0.38	0.38	0.38	0.00	0.9
4	0.37	0.24	0.31	0.10	31.0	0.21	0.19	0.20	0.01	6.5
5	0.15	<0.15	0.15	0.01	4.1	0.17	0.15	0.16	0.01	7.3
6	0.55	0.51	0.53	0.03	6.4	0.44	0.46	0.45	0.01	2.4
7	0.12	0.15	0.14	0.02	13.3	<0.15	<0.15	<0.15	/	/
8	0.18	<0.15	0.16	0.03	18.7	<0.15	<0.15	<0.15	/	/
9	0.15	0.15	0.15	0.01	4.4	0.17	<0.15	0.15	0.03	19.2
10	0.31	0.30	0.31	0.00	0.4	0.35	0.34	0.34	0.01	2.0
11	0.21	0.34	0.27	0.09	32.6	0.17	0.76	0.47	0.42	88.7
12	0.23	0.25	0.24	0.02	7.1	0.17	0.23	0.20	0.04	19.6
13	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
14	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
15	< 0.15	0.15	< 0.15	/	/	<0.15	<0.15	<0.15	/	/
16	1.18	1.10	1.14	0.06	4.9	1.07	1.06	1.06	0.01	1.2
17	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
18	0.19	0.16	0.18	0.03	14.2	0.19	<0.15	0.15	/	/
19	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
20	0.23	0.25	0.24	0.01	5.3	0.28	0.21	0.24	0.05	19.7
21	0.94	0.80	0.87	0.10	11.4	0.86	2.07	1.47	0.86	58.7
22	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
23	0.95	1.13	1.04	0.13	12.8	0.95	0.87	0.91	0.06	6.5
24	0.26	0.22	0.24	0.03	13.0	0.28	0.18	0.23	0.07	31.4
25	2.18	1.83	2.01	0.25	12.3	0.22	0.16	0.19	0.04	21.1
26	0.23	0.41	0.32	0.12	38.8	0.26	0.36	0.31	0.07	23.6
27	0.32	0.30	0.31	0.01	4.1	0.20	0.21	0.20	0.00	1.7
28	0.38	0.28	0.33	0.07	22.3	0.24	0.18	0.21	0.04	20.7
29	0.59	0.68	0.64	0.06	9.5	0.31	0.27	0.29	0.03	11.6
30	0.64	0.48	0.56	0.11	20.1	0.36	0.24	0.30	0.08	28.3
31	0.18	0.20	0.19	0.01	6.3	0.15	<0.15	0.15	0.00	2.2
32	0.23	0.25	0.24	0.01	6.0	<0.15	<0.15	<0.15	/	/
33	0.56	0.52	0.54	0.03	5.3	0.33	0.32	0.32	0.01	2.4
34	0.32	0.19	0.26	0.10	37.5	0.17	0.18	0.18	0.01	5.6
35	0.37	0.16	0.26	0.15	55.6	0.22	<0.15	0.17	0.07	38.0
36	0.95	0.17	0.56	0.55	97.8	0.16	<0.15	0.15	0.01	9.5
37	<0.15	<0.15	<0.15	/	/	<0.15	<0.15	<0.15	/	/
38	0.26	0.28	0.27	0.01	4.5	0.32	0.25	0.29	0.05	18.2
39	0.21	0.39	0.30	0.13	43.0	<0.15	<0.15	<0.15	/	/
40	0.23	0.28	0.25	0.04	14.5	0.27	0.30	0.29	0.02	7.2
41	0.49	0.35	0.42	0.09	22.4	0.34	0.29	0.32	0.04	11.6
42	0.99	0.33	0.66	0.47	71.1	<0.15	<0.15	<0.15	/	/
43	<0.15	<0.15	<0.15	/	/	0.15	0.15	0.15	0.00	1.3
44	0.41	0.40	0.41	0.01	2.3	0.40	0.36	0.38	0.03	7.3
45	0.54	0.49	0.51	0.04	7.2	0.25	0.27	0.26	0.01	3.2
46	1.07	0.75	0.91	0.22	24.6	0.23	0.25	0.24	0.01	5.3
47	0.11	0.30	0.20	0.13	64.7	<0.15	<0.15	<0.15	/	/
48	1.01	0.95	0.98	0.04	4.5	1.15	1.10	1.12	0.03	2.9

IL-1 α with Singulex

IL-1 α (pg/ml)	serum					EDTA plasma				
Sample	Det 1	Det 2	Mean	SD	CV (%)	Det 1	Det 2	Mean	SD	CV (%)
1	3.67	3.79	3.73	0.08	2.2	3.34	3.29	3.31	0.04	1.3
2	1.29	1.55	1.42	0.19	13.0	0.95	1.15	1.05	0.14	13.4
3	<0.39	0.69	0.53	0.23	42.7	<0.39	<0.39	<0.39	/	/
4	0.51	0.55	0.53	0.03	4.7	0.43	0.45	0.44	0.02	3.6
5	0.42	0.43	0.42	0.00	0.6	<0.39	<0.39	<0.39	/	/
6	0.50	0.49	0.49	0.01	1.9	<0.39	<0.39	<0.39	/	/
7	0.62	0.61	0.61	0.01	1.6	0.76	0.63	0.70	0.09	12.8
8	0.43	<0.39	<0.39	/	/	<0.39	0.61	<0.39	/	/
9	1.84	1.66	1.75	0.13	7.2	0.65	0.49	0.57	0.11	19.4
10	0.96	0.77	0.86	0.14	15.9	0.93	0.88	0.90	0.04	3.9
11	1.28	1.10	1.19	0.13	11.2	1.00	1.16	1.08	0.11	10.5
12	0.57	0.46	0.52	0.08	14.6	<0.39	0.49	0.43	0.08	19.5
13	<0.39	<0.39	<0.39	/	/	<0.39	<0.39	<0.39	/	/
14	<0.39	<0.39	<0.39	/	/	<0.39	<0.39	<0.39	/	/
15	0.62	0.51	0.56	0.08	14.0	<0.39	<0.39	<0.39	/	/
16	0.54	<0.39	<0.39	/	/	0.44	0.46	0.45	0.02	3.7
17	0.86	0.80	0.83	0.04	4.5	0.39	<0.39	<0.39	/	/
18	4.35	4.83	4.59	0.35	7.5	4.30	4.39	4.35	0.07	1.6
19	0.52	0.43	0.48	0.06	12.8	<0.39	<0.39	<0.39	/	/
20	1.28	1.15	1.22	0.10	7.9	1.57	1.29	1.43	0.20	14.0
21	<0.39	0.46	0.46	/	/	<0.39	<0.39	<0.39	/	/
22	0.57	0.56	0.57	0.01	1.6	<0.39	<0.39	<0.39	/	/
23	<0.39	<0.39	<0.39	/	/	<0.39	<0.39	<0.39	/	/
24	0.53	0.41	0.47	0.09	18.6	0.45	0.48	0.47	0.03	5.7