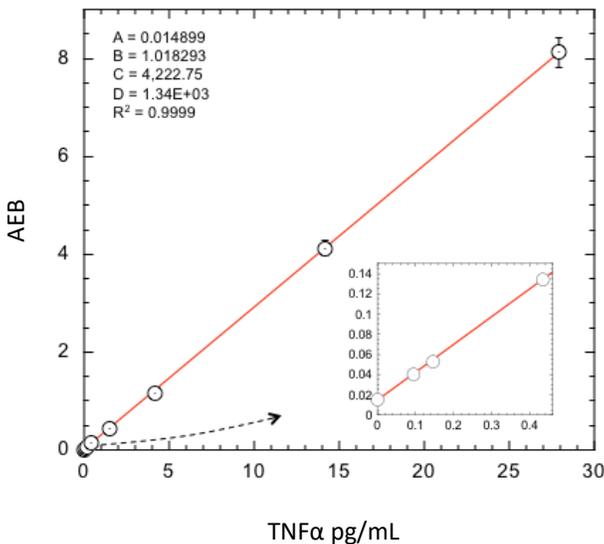


**Description**

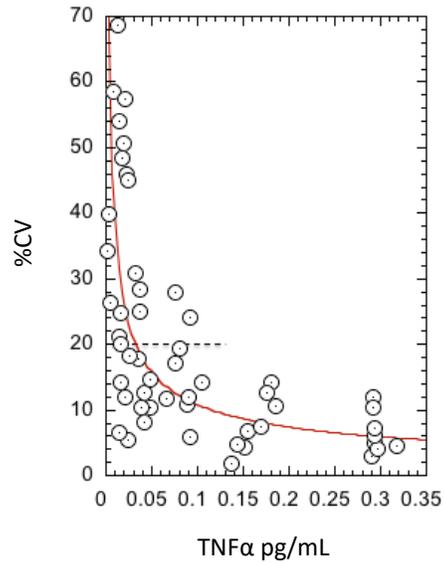
Human tumor necrosis factor alpha (TNF $\alpha$ ) is a homotrimeric transmembrane protein that functions as a proinflammatory cytokine. It is produced mainly by macrophages but also by a variety of other cell types, including monocytes, neutrophils, and T-cells. The involvement of TNF $\alpha$  in several signal transduction pathways links the protein to such diverse functions as acute inflammation, apoptosis, septic shock, cellular proliferation, and differentiation. Human TNF $\alpha$  is a non-glycosylated protein of 157 amino acids, with a molecular weight of approximately 17,000 daltons. The clinical relevance of TNF $\alpha$  stems from its association with numerous disease states including rheumatoid arthritis, cancer, cachexia, and Crohn’s disease.

**Calibration Curve:** Four-parameter curve fit parameters are depicted.



**Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 2 reagent lots across 3 instruments (22 runs total).

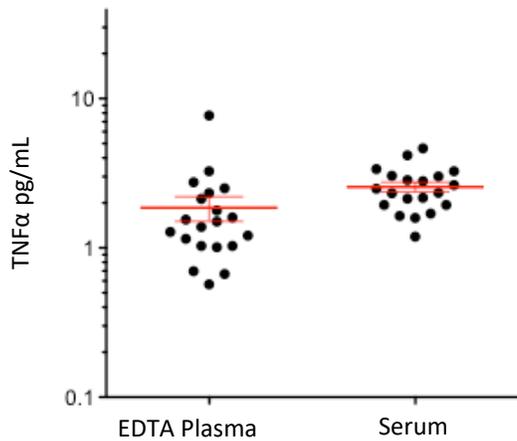
**Sample Dose CV Profile:** Triplicate measurements of diluted serum samples assayed over multiple runs (59 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit to the data.



<b>LLOQ</b>	<b>0.051 pg/mL</b>
<b>LOD</b>	<b>0.011 pg/mL</b> SD 0.0078 pg/mL
<b>Dynamic range (serum and plasma)</b>	0–112 pg/mL
<b>Diluted Sample volume*</b>	100 $\mu$ L per measurement
<b>Tests per kit</b>	96

\*See Kit Instruction for details

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=20), and serum (n=20) were measured. Error bars depict mean and SEM.



Sample Type	Median TNF $\alpha$ pg/mL	% Above LOD
Serum	2.41	100%
Plasma	1.44	100%

**Precision:** Five samples consisting of two serum-based panels, one plasma-based panel, and two TNF $\alpha$  controls were assayed in replicates of three at two separate times per day for five days using a single stored calibration curve and a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	1.80	5.7%	3.7%	6.2%
Control 2	43.3	7.9%	5.5%	4.7%
Panel 1	1.81	5.6%	5.7%	8.0%
Panel 2	4.69	5.8%	5.4%	4.7%
Panel 3*	4.69	4.2%	6.1%	0.0%

\*Plasma

**Spike and Recovery:** TNF $\alpha$  spiked into 4 serum samples at 4 and 40 pg/mL.

**Admixture Linearity:** High TNF $\alpha$  sample admixed with low TNF $\alpha$  sample, mean of 12 levels.

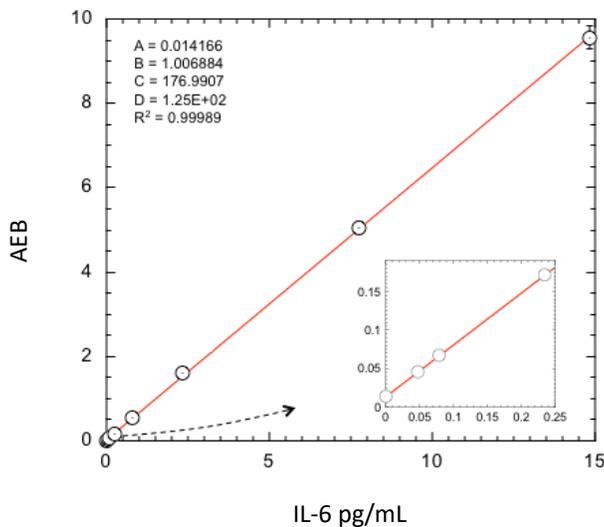
**Dilution Linearity:** Serum sample diluted 2x serially from MRD (4x) to 256x with sample diluent.

<b>Spike and Recovery (Serum)</b>	<b>Mean = 96.5%</b> Range: 80.5–111.8%
<b>Admixture Linearity</b>	<b>Mean = 105.6%</b> Range: 100.0–112.3%
<b>Dilution Linearity (256x)</b>	<b>Mean = 69.1%</b> Range: 63.2–75.1%

**Description**

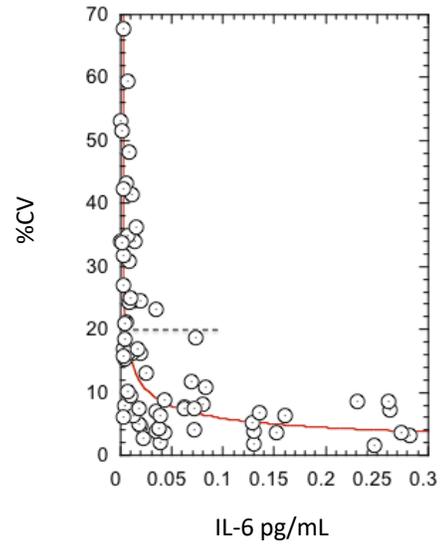
Interleukin 6 (IL-6) is an alpha-helical cytokine with a wide variety of biological functions, including inducement of acute phase reactions, inflammation, hematopoiesis, bone metabolism, and cancer progression. It is secreted by multiple cell types as a 22kDa-28kDa phosphorylated and variably glycosylated molecule. Mature human IL-6 is 183 amino acids (aa) in length and shares 41% aa sequence identity with mouse and rat IL-6. IL-6 is secreted by T cells and macrophages to induce immune responses following tissue trauma leading to inflammation. IL-6 also acts as an anti-inflammatory myokine, secreted by muscles during contraction after which it acts to increase breakdown of fats and improve insulin resistance. Because of its role in inducing inflammation and auto-immune response, there is interest in developing anti-IL-6 agents as potential therapies against various diseases, including rheumatoid arthritis and cancer.

**Calibration Curve:** Four-parameter curve fit parameters are depicted.



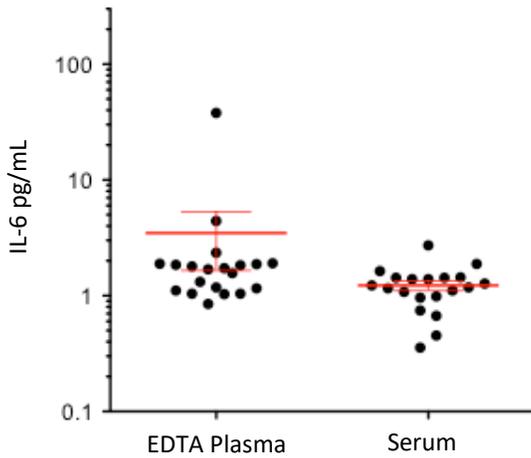
**Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 2 reagent lots across 3 instruments (22 runs total).

**Sample Dose CV Profile:** Triplicate measurements of diluted serum samples assayed over multiple runs (71 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit to the data.



<b>LLOQ</b>	<b>0.011 pg/mL</b>
<b>LOD</b>	<b>0.006 pg/mL</b> SD 0.0035 pg/mL
<b>Dynamic range (serum and plasma)</b>	0–60 pg/mL
<b>Diluted Sample volume*</b>	100 $\mu$ L per measurement
<b>Tests per kit</b>	96

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=20), and serum (n=20) were measured. Error bars depict mean and SEM.



**Spike and Recovery:** IL-6 spiked into 4 serum samples at 2 and 20 pg/mL.

**Admixture Linearity:** High IL-6 sample admixed with low IL-6 sample, mean of 12 levels.

**Dilution Linearity:** Serum sample diluted 2x serially from MRD (4x) to 256x with Sample Diluent.

<b>Spike and Recovery (Serum)</b>	<b>Mean = 97.5%</b> Range: 84.1–116.8%
<b>Admixture Linearity</b>	<b>Mean = 101.0%</b> Range: 95.7–106.8%
<b>Dilution Linearity (256x)</b>	<b>Mean = 88.0%</b> Range: 80.0–97.7%

Sample Type	Median IL-6 pg/mL	% Above LOD
Serum	1.21	100%
Plasma	1.71	100%

**Precision:** Five samples consisting of two serum-based panels, one plasma-based panel, and two IL-6 controls were assayed in replicates of three at two separate times per day for five days using a single stored calibration curve and a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

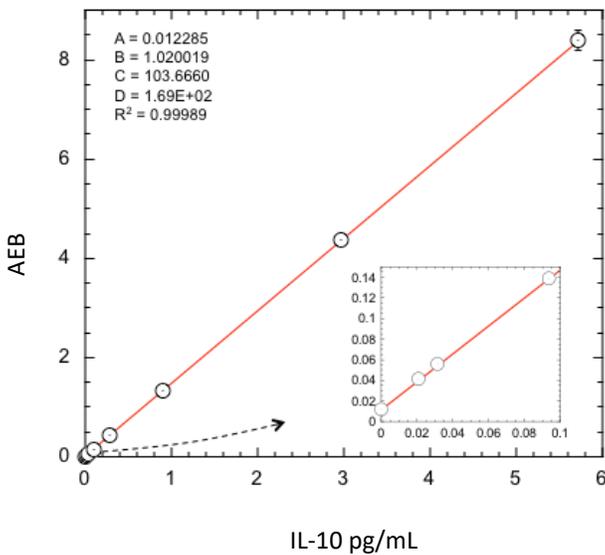
Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	0.966	5.0%	6.0%	6.4%
Control 2	23.6	7.5%	6.3%	5.6%
Panel 1	19.7	4.3%	6.7%	6.6%
Panel 2	1.09	6.4%	5.2%	5.8%
Panel 3*	2.27	5.3%	4.5%	3.6%

\*Plasma

**Description**

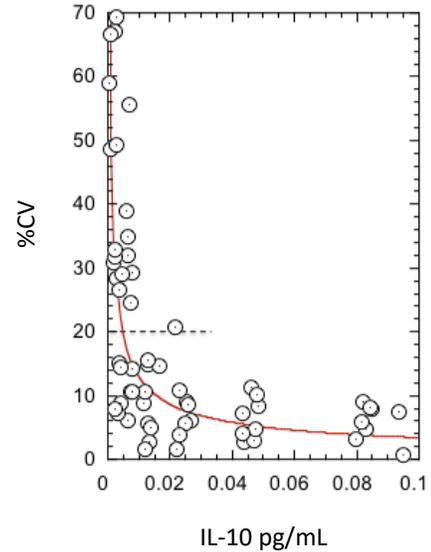
Interleukin 10 (IL-10) is an alpha-helical, homodimeric cytokine, each subunit composed of 178 amino acids (18 kDa). The major role of IL-10 is to act as an anti-inflammatory cytokine. It is produced primarily by monocytes, type 2 T helper cells and B cells. IL-10 is also released by cytotoxic T cells to inhibit the action of NK cells during the immune response to viral infection. It has multiple effects in immunoregulation and inflammation, including down regulation of Th1 cytokine expression, MHC class II antigens, and stimulatory molecules on macrophages. IL-10 can also inhibit synthesis of pro-inflammatory cytokines such as IFN-g, IL-2, TNF $\alpha$  and GM-CSF made by macrophages and regulatory T cells. IL-10 is among cytokines secreted by muscle cells, whose elevation during physical activity suggests that exercise promotes an environment of anti-inflammatory cytokines. IL-10 has garnered interest as a potential anti-inflammatory therapeutic, but initial studies with rheumatoid arthritis have shown limited efficacy.

**Calibration Curve:** Four-parameter curve fit parameters are depicted.



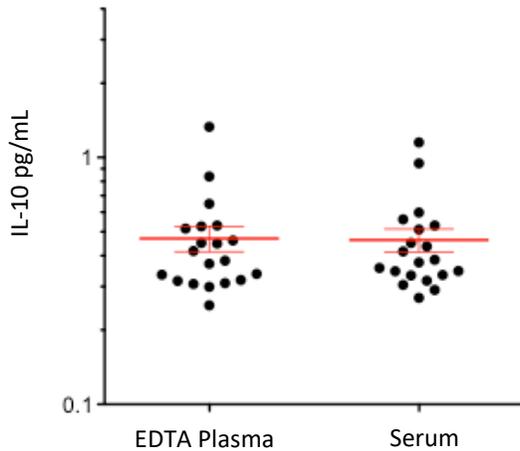
**Lower Limit of Detection (LOD):** Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 2 reagent lots across 3 instruments (22 runs total).

**Sample Dose CV Profile:** Triplicate measurements of diluted serum samples assayed over multiple runs (64 measurements). LLOQ determined as the concentration at which %CV exceeds 20% according to the power equation fit to the data.



<b>LLOQ</b>	<b>0.0073 pg/mL</b>
<b>LOD</b>	<b>0.0022 pg/mL</b> SD 0.00118 pg/mL
<b>Dynamic range (serum and plasma)</b>	0–24 pg/mL
<b>Diluted Sample volume*</b>	100 $\mu$ L per measurement
<b>Tests per kit</b>	96

**Endogenous Sample Reading:** Healthy donor matched EDTA plasma (n=20) and serum (n=20) were measured. Error bars depict mean and SEM.



**Spike and Recovery:** IL-10 spiked into 4 serum samples at 0.78, and 7.8 pg/mL.

**Admixture Linearity:** High IL-10 sample admixed with low IL-10 sample, mean of 12 levels.

**Dilution Linearity:** Serum sample diluted 2x serially from MRD (4x) to 256x with Sample Diluent.

Spike and Recovery (Serum)	Mean = 85.8% Range: 72.9–97.0%
Admixture Linearity	Mean = 97.0% Range: 86.8–103.2%
Dilution Linearity (256x)	Mean = 89.5% Range: 80.3–98.4%

The Simoa Cytokine 3-Plex A assay kit is formulated for use on the SR-X®, HD-1, or HD-X® platform. Data in this document was obtained from runs on the HD-1 platform unless otherwise noted. Some differences in performance claims between SR-X and HD-1/HD-X may be observed when comparing datasheets for these platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or it may be due to minor differences in antibody and analyte behavior in the different assay formats.

Sample Type	Median IL-10 pg/mL	% Above LOD
Serum	0.381	100%
Plasma	0.400	100%

**Precision:** Five samples consisting of two serum-based panels, one plasma-based panel, and two IL-10 controls were assayed in replicates of three at two separate times per day for five days using a single stored calibration curve and a single lot of reagents. Analysis of variance (fully nested ANOVA) results are summarized in the following table.

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between day CV
Control 1	0.492	3.9%	5.3%	5.7%
Control 2	11.4	7.2%	6.8%	4.6%
Panel 1	0.428	5.6%	6.1%	5.6%
Panel 2	0.377	6.4%	5.0%	2.4%
Panel 3*	0.470	4.7%	5.5%	0.0%

\*Plasma