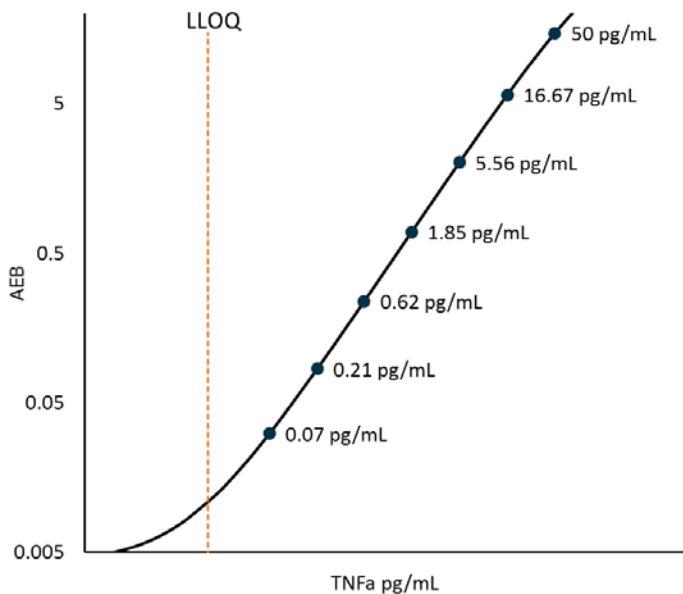


Description

Human tumor necrosis factor alpha (TNFα) 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 TNFα in several signal transduction pathways links the protein to such diverse functions as acute inflammation, apoptosis, septic shock, cellular proliferation, and differentiation. Human TNFα is a non-glycosylated protein of 157 amino acids, with a molecular weight of approximately 17,000 daltons. The clinical relevance of TNFα stems from its association with numerous disease states including rheumatoid arthritis, cancer, cachexia, and Crohn’s disease.

Calibration Curve: Calibrator concentrations and Lower Limit of Quantification depicted.



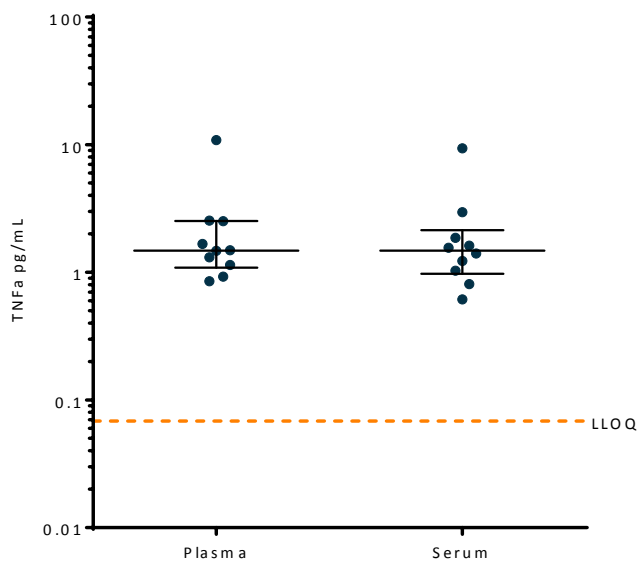
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

LLOQ	0.0171 pg/mL pooled CV 11% mean recovery 111%
LOD	0.0039 pg/mL range 0.0010-0.0100 pg/mL
Dynamic range	0–200 pg/mL
Diluted Sample volume (1:4 Dilution)*	100 μL per measurement
Tests per kit	96

*See Kit Instruction for details

Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=10) and serum (n=10) samples were measured. Bars depict median with interquartile range. Orange line represents functional LLOQ.



Sample Type	Mean TNFa pg/mL	Median TNFa pg/mL	% Above LOD
EDTA plasma	2.48	1.48	100%
Serum	2.24	1.48	100%

Precision: Measurements of 3 serum or plasma based panels and 2 calibrator based controls. Triplicate measurements were made for 3 runs each for 1 reagent lot across 2 instruments (6 runs total, 18 measurements).

Sample	Mean (pg/mL)	Within run CV	Between run CV	Between inst CV
Control 1	2.43	7.1%	8.7%	4.7%
Control 2	44.3	5.2%	5.3%	0.8%
Panel 1	12.8	4.9%	18.1%	5.4%
Panel 2	1.75	4.5%	9.7%	5.2%
Panel 3	1.77	5.0%	6.6%	2.1%

Dilution Linearity: 1 spiked EDTA plasma sample and 1 spiked serum sample were serially diluted in sample diluent from 2X to 128X and run neat on the instrument.

Spiked Plasma (128x)	Mean = 121% Range: 86-135%
Spiked Serum (128x)	Mean = 111% Range: 100-124%

Note: Spike and Recovery data was obtained using the HD-1 Analyzer.

Spike and Recovery: 2 EDTA plasma samples and 2 serum samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

Spike and Recovery	78% Range 68-87%
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The Simoa TNFa assay kit is formulated for use on either the SR-X or HD-1 platform. Minor differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two different platforms, due to experiments run at different time-points with different reagent lots and different samples. Data in this document was obtained from runs on the SR-X platform unless otherwise noted.