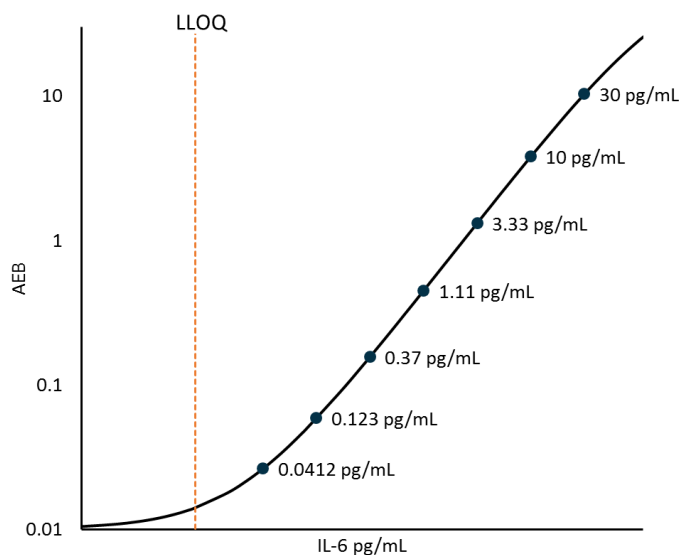


Description

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 22-28kD 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: Calibrator concentrations and Lower Limit of Quantification depicted.



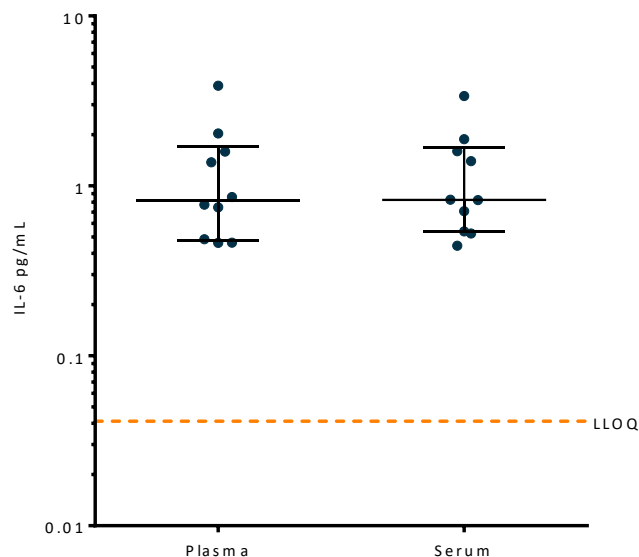
Lower Limit of Quantification (LLOQ): Triplicate measurements of serially diluted calibrator were read back on the calibration curve over 3 runs each for 1 reagent lot across 2 instruments (6 runs total).

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 | 0.0103 pg/mL pooled CV 18% mean recovery 95% |
| LOD | 0.0062 pg/mL range 0.0040-0.0088 pg/mL |
| Dynamic range | 0–120 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.



| Sample Type | Mean IL-6 pg/mL | Median IL-6 pg/mL | % Above LOD |
|-------------|-----------------|-------------------|-------------|
| EDTA plasma | 1.27 | 1.58 | 100% |
| Serum | 1.21 | 1.60 | 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 | 1.67 | 4.2% | 3.6% | 3.7% |
| Control 2 | 25.3 | 6.4% | 6.0% | 1.6% |
| Panel 1 | 48.4 | 6.1% | 5.0% | 1.5% |
| Panel 2 | 5.18 | 4.0% | 4.2% | 1.2% |
| Panel 3 | 1.80 | 4.0% | 5.8% | 3.4% |

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

Spike and Recovery: 4 serum samples were spiked at high and low concentrations within the range of the assay and analyzed on HD-1.

Dilution Linearity: 1 endogenous plasma sample and 1 spiked serum sample were diluted 2X serially from 4x (MRD) to 128x with Sample Diluent.

| | |
|---|---------------------------------------|
| Spike and Recovery | 83% Range 69-98% |
| Endogenous Dilution Linearity (128x) | Mean = 120% Range: 112-125% |
| Spiked Dilution Linearity (128x) | Mean = 108% Range: 98-112% |

The Simoa IL-6 assay kit is formulated for use on either the SR-X or HD-1 platform. Data in this document was obtained from runs on the SR-X platform unless otherwise noted. Some differences in performance claims between the HD-1 and SR-X may be observed when comparing datasheets for the two platforms. This may be due to experiments run at different time-points with different reagent lots and different samples, or may be due to minor differences in antibody and analyte behavior in the different assay formats.