Simoa® IL-5 Advantage PLUS Kit
HD-X Data Sheet

Item 105187

Description

This data sheet summarizes data from the analytical validation performed at Quanterix to characterize performance of the IL-5 Advantage PLUS kit on the HD-X platform.

IL-5

Interleukin 5 (IL-5) is a cytokine with a length of 115 amino acids and a molecular weight of 15.2 kDa that is derived from T-cells with hematopoietic functions predominantly associated with antigen-induced eosinophilia. IL-5 induces differentiation of B-cells to immunoglobulin secreting cells and is an important factor in growth, differentiation and activation of eosinophils. IL-5, GM-CSF, and IL-3 comprise the β-common (βc) cytokine family, so named because the receptors share a common β chain complexed with cytokine-specific α chains. Activation of the IL-3/IL-5/GM-CSF receptors results in rapid activation of the JAK/STAT pathway. IL-5 and IL-5 receptor are the targets of therapeutic antibodies for treatment of eosinophilic asthma and are involved in type 2 inflammation in the mucosal allergic reaction to grass pollen.

Calibration Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted in Figure 1. The assigned concentrations of calibrator levels and reconstitution volume may vary between different kit lots.

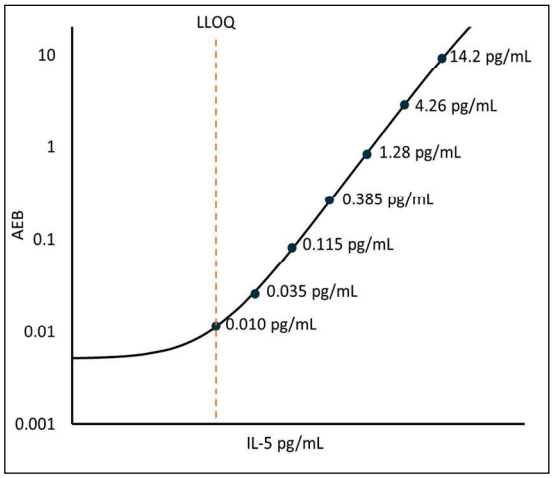

Figure 1. Example calibrator curve.

Minimum Required Dilution (MRD)

Diluted Sample Volume	100 µL per measurement
Serum and EDTA Plasma Dilution	1:4
Tests per Kit	96

See Kit Instruction for details.

Lower Limit of Quantification (LLOQ): The analytical LLOQ was set at the lowest concentration that read back within 80 – 120% of the expected value with a CV ≤ 20%. The functional LLOQ (fLLOQ) values below are for serum and EDTA plasma and represent the analytical LLOQ multiplied by the dilution factor used for the samples.

Limit of Detection (LOD): Calculated as 2.5 standard deviations from the mean of background signal read back on each calibration curve.

Assay Range: The upper end of the dynamic range is equal to the top calibrator concentration multiplied by MRD. The representative ranges below are for serum and EDTA plasma.

IL-5	
Analytical LLOQ	0.01 pg/mL Pooled CV: 20% Mean Recovery: 103%
Functional LLOQ	Serum/EDTA Plasma (4x): 0.04 pg/mL
LOD	0.002 pg/mL Range: 0.001 - 0.003 pg/mL
Dynamic Range	Serum/EDTA Plasma (4x): 0 - 60 pg/mL

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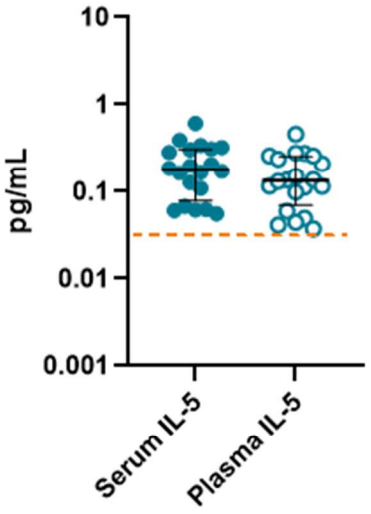
Endogenous Sample Reading: Healthy donor matched EDTA plasma (n=20), and serum (n=20) concentrations (pg/mL) were measured using the IL-5 Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange line represents functional LLOQ.



IL-5				
Sample Type	Mean (pg/mL)*	Median (pg/mL)*	% Above LOD	% Above LLOQ
Serum	0.21	0.178	100%	100%
EDTA Plasma	0.167	0.136	100%	95%

*Values below LLOQ are excluded from the mean and median calculation.

Precision: Measurements of 2 calibrator-based controls, 3 commercial pooled serum and 3 commercial pooled plasma. Triplicate measurements were made for 2 runs each for 1 reagent lot across 2 instruments (4 runs total, 12 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

IL-5				
Sample	Mean (pg/mL)	Within Run CV	Between Run CV	Between Instr CV
Control 1	0.756	3.3%	4.2%	3.1%
Control 2	11.9	3.9%	7.9%	3.4%
Plasma 1	0.147	13%	19%	9.9%
Plasma 2	34.4	5.5%	3.3%	1.9%
Plasma 3	29.2	6.7%	4.0%	2.8%
Serum 1	0.584	7.4%	8.9%	1.4%
Serum 2	35.5	7.6%	5.6%	0.7%
Serum 3	35.6	4.4%	14%	3.0%

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-5 within the range of each assay and analyzed on HD-X. Percent recovery is defined as the difference between the measured concentration of the analytes in the spiked sample and the measured concentration in unspiked sample relative to the concentration of the analytes in spiked calibrator diluent.

Dilution Linearity: 2 serum and 2 EDTA plasma samples were spiked with endogenous antigen and then diluted 2x serially with sample diluent. Total dilution of each sample ranged from 4x to 64x.

IL-5 †	
Spike and Recovery Serum	Mean: 85% Range: 79 – 90%
Spike and Recovery EDTA Plasma	Mean: 82% Range: 72 – 92%
Dilution Linearity Serum (4X-64X)	Mean: 105% Range: 97 - 115%
Dilution Linearity EDTA Plasma (4X-64X)	Mean: 106% Range: 92 - 136%

† The assay design is conserved between C4PB Advantage PLUS and IL-5 Advantage PLUS. Claims are from C4PB Advantage PLUS Validation.

The Simoa® IL-5 Advantage PLUS assay kit is formulated for use on the HD-X platform. Verification and validation results for the fully automated HD-X instrument are summarized in this report.

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