

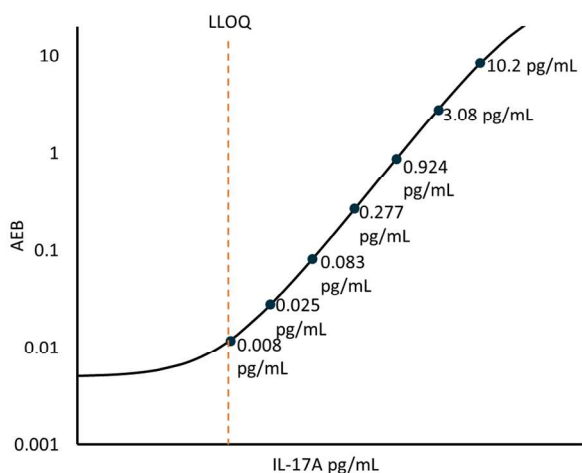
Description

This datasheet summarizes data from analytical validation performed at Quanterix to characterize performance of the Cytokine 4-Plex B (C4PB) kit on the HD-X platform. Data provided includes Calibration Curves, Minimum Required Dilution (MRD), Lower Limit of Qualification (LLOQ), Limit of Detection (LOD), Assay Range, Precision, Spike and Recovery, and Dilution Linearity.

IL-17A

Interleukin 17A (IL-17A) is disulfide-linked in homodimeric cytokine of 155 amino acids (molecular weight 35 kDa) and a member of an IL-17 family of related cytokines (IL-17B through IL-17F). All IL-17A cytokines have a similar protein structure, and no sequence similarity to any other cytokines. These cytokines are well conserved in mammals, with significant sequence conservation between the human and mouse homologs. A major role of IL-17A is its involvement in inducing and mediating proinflammatory responses. It acts as a potent mediator in delayed-type reactions by increasing chemokine production in various tissues to recruit monocytes and neutrophils to the site of inflammation, like interferon gamma. The IL-17A family has been linked to many immune/autoimmune related diseases including rheumatoid arthritis, asthma, lupus, allograft rejection, anti-tumor immunity and recently psoriasis.

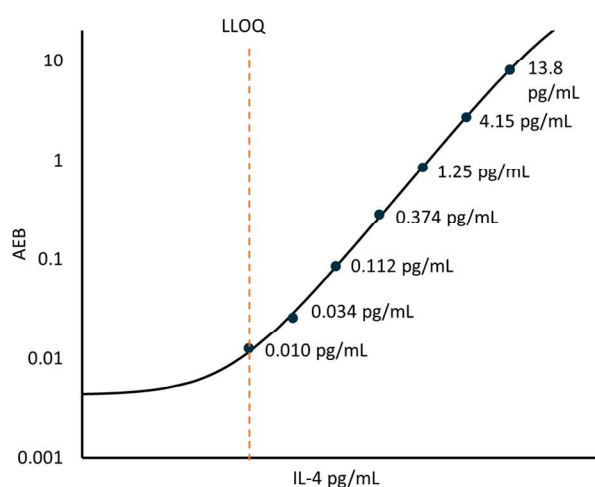
IL-17A Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



IL-4

Interleukin 4 (IL-4) is a monomeric cytokine, approximately 13-18 kDa, expressed by T helper (Th) 2 biased CD4+ T cells, mast cells, basophils, and eosinophils. IL-4 has a compact, globular fold (similar to other cytokines) stabilized by 3 disulfide bonds which are crucial for its bioactivity. Mature human IL-4 shares 55%, 39%, and 43% amino acid sequence identity with bovine, mouse, and rat IL-4, respectively. By binding to IL-4 receptor or receptor complex, IL-4 has many biological functions. It promotes cell proliferation, survival, and immunoglobulin class switch to IgG4 and IgE in human B cells, acquisition for the Th2 phenotype by naïve CD4+ T cells, priming and chemotaxis of mast cells, eosinophils and basophils, and the proliferation and activation of epithelial cells. IL-4 plays an important role in the development of allergic inflammation, asthma and autoimmune diseases.

IL-4 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



IL-13

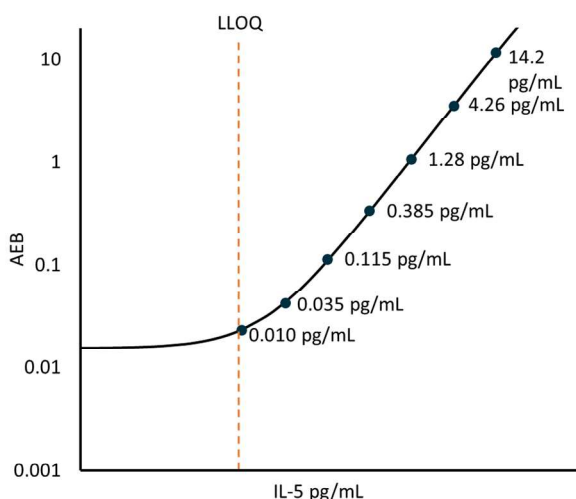
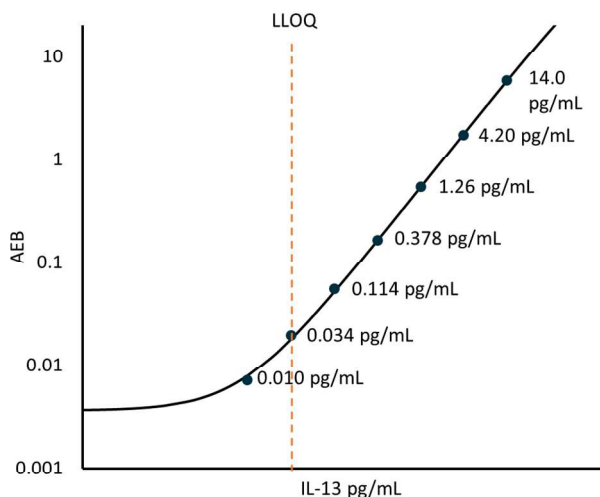
Interleukin 13 (IL-13) is a cytokine of 111 amino acids (molecular weight 15.8 kDa) whose major roles include down-modulation of macrophage activity (lowering the production of pro-inflammatory cytokines) and mediation of allergic responses. It is secreted by many cell types, but primarily by activated T cells, in particular T helper type 2 cells. IL-13 affects immune cells in a manner similar to IL-4 but it is more associated with physiological changes induced through a receptor that includes the alpha chain of the IL-4 receptor and at least one or two known IL-13 specific binding chains. IL-13 is the central mediator of allergic asthma, where it regulates eosinophilic inflammation, mucus secretion, and airway hyperresponsiveness. IL-13 has therefore become a therapeutic target for allergic diseases with several anti-IL-13 antibodies under evaluation as treatment for bronchial asthma. Manipulation of IL-13 effector function may also prove useful in the treatment of some cancers like B-cell chronic lymphocytic leukemia and Hodgkin’s disease, where IL-13 modulates apoptosis or tumor cell growth.

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.

IL-13 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.

IL-5 Curve: Representative calibrator concentrations and Lower Limit of Quantification (LLOQ) depicted.



Minimum Required Dilution (MRD)

| | |
|----------------------------|---------------------------|
| Diluted Sample Volume | 100 µL per measurement |
| Serum/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 over 6 runs each for 2 reagent lot across 2 instruments (3 runs per lot, per instrument, 12 runs total).

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.

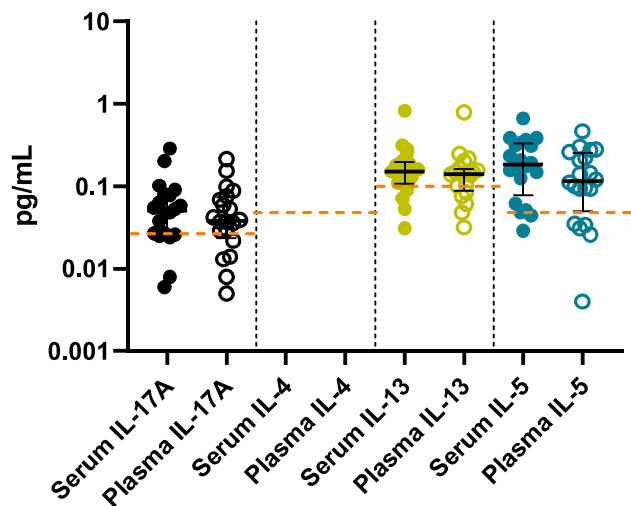
| IL-4 | |
|-----------------|---|
| Analytical LLOQ | 0.01 pg/mL Pooled CV: 18% Mean Recovery: 102% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.04 pg/mL |
| LOD | 0.003 pg/mL Range: 0.001 - 0.007 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 40 pg/mL |

| IL-13 | |
|-----------------|--|
| Analytical LLOQ | 0.034 pg/mL Pooled CV: 10% Mean Recovery: 101% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.136 pg/mL |
| LOD | 0.004 pg/mL Range: 0.001 - 0.007 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 60 pg/mL |

| IL-17A | |
|-----------------|---|
| Analytical LLOQ | 0.008 pg/mL Pooled CV: 18% Mean Recovery: 94% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.032 pg/mL |
| LOD | 0.002 pg/mL Range: 0.001 - 0.003 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 48 pg/mL |

| IL-5 | |
|-----------------|---|
| Analytical LLOQ | 0.01 pg/mL Pooled CV: 18% Recovery: 99% |
| Functional LLOQ | Serum/EDTA Plasma (4x): 0.04 pg/mL |
| LOD | 0.004 pg/mL Range: 0.001 - 0.008 pg/mL |
| Dynamic Range | Serum/EDTA Plasma (4x): 0 - 60 pg/mL |

Endogenous Sample Reading: Concentrations (pg/mL) were determined for matched EDTA plasma (n=20), and serum (n=20) from normal human donors using the C4PB Advantage PLUS kit on HD-X. Bars depict median with interquartile range. Orange line represents functional LLOQ.



| IL-13 | | | | |
|-------------|--------------|----------------|-------------|--------------|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 0.254 | 0.186 | 100% | 60% |
| EDTA Plasma | 0.214 | 0.148 | 100% | 65% |

| IL-5 | | | | |
|-------------|--------------|----------------|-------------|--------------|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 0.23 | 0.191 | 100% | 95% |
| EDTA Plasma | 0.194 | 0.144 | 95% | 75% |

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

Precision: Measurements of 2 calibrator-based controls, 3 commercial pooled serum panels and 3 commercial pooled plasma panels. Triplicate measurements were made for 6 runs each for 2 reagent lot across 2 instruments (12 runs total, 36 measurements). All samples were diluted at the appropriate MRD for the sample matrix.

| IL-17A | | | | |
|-------------|--------------|----------------|-------------|--------------|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | 0.093 | 0.064 | 90% | 65% |
| EDTA Plasma | 0.077 | 0.059 | 95% | 65% |

| IL-17A | | | | | |
|-----------|--------------|---------------|----------------|----------------|------------------|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 0.529 | 4.4% | 7.9% | 0.7% | 2.5% |
| Control 2 | 8.56 | 2.5% | 5.0% | 0.4% | 0.2% |
| Panel 1 | 0.141 | 8.0% | 13% | 0.3% | 4.5% |
| Panel 2 | 6.59 | 3.6% | 8.6% | 6.2% | 0.7% |
| Panel 3 | 6.50 | 5.9% | 12% | 2.6% | 2.5% |
| Panel 4 | 0.126 | 7.5% | 11% | 1.8% | 6.6% |
| Panel 5 | 6.84 | 5.4% | 11% | 2.5% | 4.4% |
| Panel 6 | 6.46 | 5.7% | 15% | 0.7% | 1.0% |

| IL-4 | | | | |
|-------------|--------------|----------------|-------------|--------------|
| Sample | Mean (pg/mL) | Median (pg/mL) | % Above LOD | % Above LLOQ |
| Serum | <LOD | <LOD | 0% | 0% |
| EDTA Plasma | <LOQ | <LOQ | 5-45% | 0% |

| IL-4 | | | | | |
|-----------|--------------|---------------|----------------|----------------|------------------|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 0.724 | 4.4% | 6.2% | 3.5% | 4.6% |
| Control 2 | 11.9 | 2.2% | 3.9% | 0.2% | 0.8% |
| Panel 1 | 0.370 | 5.8% | 12% | 3.6% | 5.6% |
| Panel 2 | 10.7 | 3.3% | 7.5% | 3.8% | 0.6% |
| Panel 3 | 10.6 | 5.5% | 10% | 5.0% | 3.0% |
| Panel 4 | 0.402 | 7.5% | 11% | 3.3% | 7.1% |
| Panel 5 | 10.0 | 5.5% | 8.4% | 1.6% | 3.5% |
| Panel 6 | 10.0 | 5.2% | 14% | 0.1% | 0.7% |

| IL-13 | | | | | |
|-----------|--------------|---------------|----------------|----------------|------------------|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 0.723 | 6.1% | 6.2% | 2.2% | 3.3% |
| Control 2 | 11.8 | 3.7% | 5.8% | 2.3% | 2.2% |
| Panel 1 | 0.448 | 5.8% | 14% | 13% | 4.1% |
| Panel 2 | 14.2 | 3.0% | 8.7% | 4.3% | 4.5% |
| Panel 3 | 14.8 | 5.8% | 12% | 2.7% | 3.0% |
| Panel 4 | 0.268 | 8.0% | 11% | 5.4% | 6.1% |
| Panel 5 | 7.87 | 4.4% | 10% | 1.9% | 6.9% |
| Panel 6 | 7.37 | 4.2% | 14% | 0.05% | 5.7% |

| IL-5 | | | | | |
|-----------|--------------|---------------|----------------|----------------|------------------|
| Sample | Mean (pg/mL) | Within Run CV | Between Run CV | Between Lot CV | Between Instr CV |
| Control 1 | 0.737 | 3.7% | 6.5% | 1.0% | 4.8% |
| Control 2 | 12.2 | 2.5% | 5.6% | 0.4% | 1.9% |
| Panel 1 | 0.167 | 6.4% | 14% | 2.8% | 6.7% |
| Panel 2 | 33.9 | 3.3% | 8.8% | 7.2% | 1.7% |
| Panel 3 | 27.7 | 5.5% | 12% | 2.9% | 4.0% |
| Panel 4 | 0.554 | 6.5% | 12% | 4.5% | 11% |
| Panel 5 | 32.2 | 6.0% | 10% | 3.8% | 5.5% |
| Panel 6 | 32.0 | 5.6% | 14% | 1.3% | 1.6% |

Spike and Recovery: 2 serum and 2 EDTA plasma samples were spiked at high and low concentrations of IL-17A, IL-4, IL-13, and 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 serially diluted 2X with sample diluent and then tested at 2XMRD. Total dilution of each sample ranged from 4x to 64x. For valid comparison between results, it is recommended to run all samples at a consistent dilution.

| IL-17A | |
|---|---------------------------------|
| Spike and Recovery Serum | Mean: 81% Range: 77 – 84% |
| Spike and Recovery EDTA Plasma | Mean: 76% Range: 69 – 85% |
| Dilution Linearity Serum (4X-64X) | Mean: 114% Range: 105 - 129% |
| Dilution Linearity EDTA Plasma (4X-64X) | Mean: 111% Range: 104 - 126% |

| IL-4 | |
|---|---------------------------------|
| Spike and Recovery Serum | Mean: 82% Range: 77 - 91% |
| Spike and Recovery EDTA Plasma | Mean: 85% Range: 79 – 90% |
| Dilution Linearity Serum (4X-64X) | Mean: 110% Range: 96 - 126% |
| Dilution Linearity EDTA Plasma (4X-64X) | Mean: 107% Range: 100 - 128% |

| IL-13 | |
|---|---------------------------------|
| Spike and Recovery Serum | Mean: 77% Range: 73 – 82% |
| Spike and Recovery EDTA Plasma | Mean: 82% Range: 76 – 87% |
| Dilution Linearity Serum (4X-64X) | Mean: 117% Range: 103 – 133% |
| Dilution Linearity EDTA Plasma (4X-64X) | Mean: 104% Range: 91 – 118% |

| 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 Simoa Cytokine 4-Plex B (C4PB) 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.