

# SR-X Compatibility for Simoa® Cytokine 4-plex B Advantage PLUS Assay

## Overview

This document describes compatibility of the Cytokine 4-plex A (C4PB) Advantage PLUS assay (Item #104997) on the Quanterix SR-X® platform. This compatibility report aims to complement and expand upon the validation data presented in the C4PB Advantage PLUS Validation Report, available on the Quanterix® customer portal<sup>1</sup>. The validation report details analytical assay performance, including LLOQ, LOD, precision, spike and recovery, dilution linearity and measurement of normal samples. Compatibility of the C4PB Advantage PLUS assay was demonstrated through comparative analysis of the assay's performance across the HD-X and SR-X platforms using a sub-set of parameters from the validation report. Further validation of assay performance may be required prior to initiating large projects with the assay on the SR-X platform, depending on specific in-house regulations. Users should build familiarity with the workflow for the assay kits on the SR-X, following the Kit Instructions provided, for optimal results. If further information is needed, please contact [Techsupport@quanterix.com](mailto:Techsupport@quanterix.com).

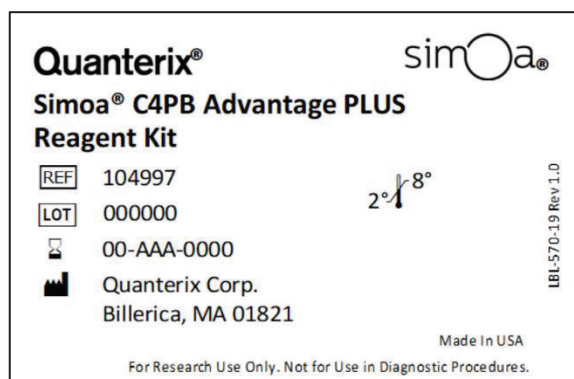
## Assay Performance

The analytical performance of C4PB Advantage PLUS assay on the Simoa HD-X Analyzer® platform can be obtained from the Data sheet available on the Quanterix customer portal<sup>2</sup>. Tables 1-4 compare the quantitation limit and the detection limit and Figures 1-4 provide curve comparisons and sample reading correlation between HD-X and SR-X platforms for C4PB Advantage PLUS.

Tables 1-4. Quantification and detection limits for C4PB Advantage PLUS assays across HD-X and SR-X platforms. Refer to the assay specific data sheet for additional details. Triplicate measurements of serially diluted calibrator were read back on the calibration curve over two runs each for one reagent lot across two instruments on SR-X platform. Twenty matched EDTA plasma and serum samples were tested for detectability and quantifiability.

Assay Parameters Comparison IL-17A (C4PB)		
	HD-X as per Data Sheet	SR-X
Curve profiling LLOQ	0.0080 pg/mL	0.0128 pg/mL
Functional LLOQ (Analytical LLOQ x MRD)	0.0320 pg/mL	0.0510 pg/mL
LOD	0.0021 pg/mL	0.0030 pg/mL

Assay Parameters Comparison IL-4 (C4PB)		
	HD-X	SR-X
Curve profiling LLOQ	0.0100 pg/mL	0.0183 pg/mL
Functional LLOQ (Analytical LLOQ x MRD)	0.0400 pg/mL	0.0730 pg/mL
LOD	0.0031 pg/mL	0.0036 pg/mL



#### Assay Parameters Comparison IL-13 (C4PB)

	HD-X	SR-X
Curve profiling LLOQ	0.0340 pg/mL	0.0230 pg/mL
Functional LLOQ (Analytical LLOQ x MRD)	0.1360 pg/mL	0.0920 pg/mL
LOD	0.0039 pg/mL	0.0042 pg/mL

#### Assay Parameters Comparison IL-5 (C4PB)

	HD-X	SR-X
Curve profiling LLOQ	0.0100 pg/mL	0.0178 pg/mL
Functional LLOQ (Analytical LLOQ x MRD)	0.0400 pg/mL	0.0710 pg/mL
LOD	0.0044 pg/mL	0.0036 pg/mL

### I. Calibrator Comparison

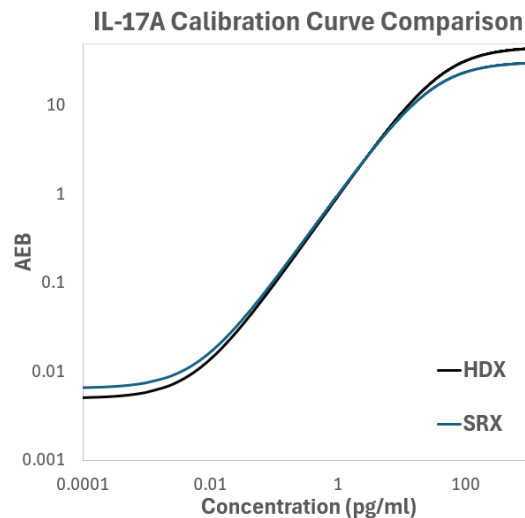


Figure 1. Comparison of the calibration curves for IL-17A in C4PB Advantage PLUS kits across HD-X (black) and SR-X (blue) platforms for one reagent kit lot.

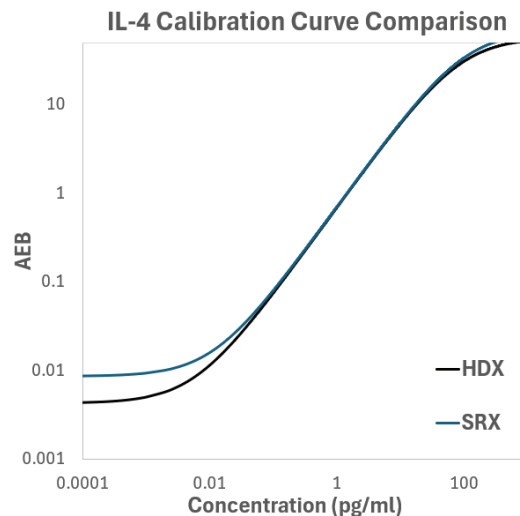


Figure 2. Comparison of the calibration curves for IL-4 in C4PB Advantage PLUS kits across HD-X (black) and SR-X (blue) platforms for one reagent kit lot.

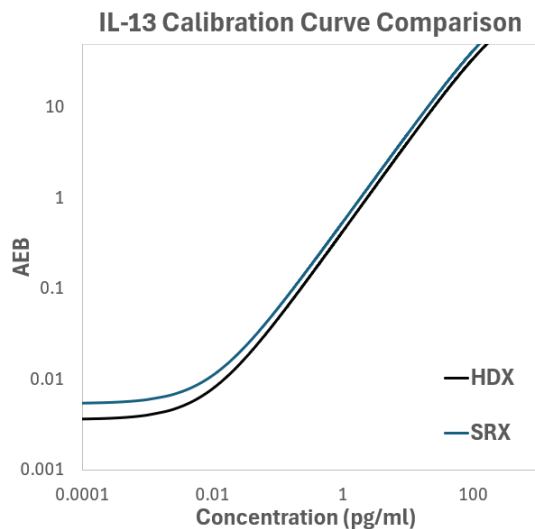


Figure 3. Comparison of the calibration curves for IL-13 in C4PB Advantage PLUS kits across HD-X (black) and SR-X (blue) platforms for one reagent kit lot.

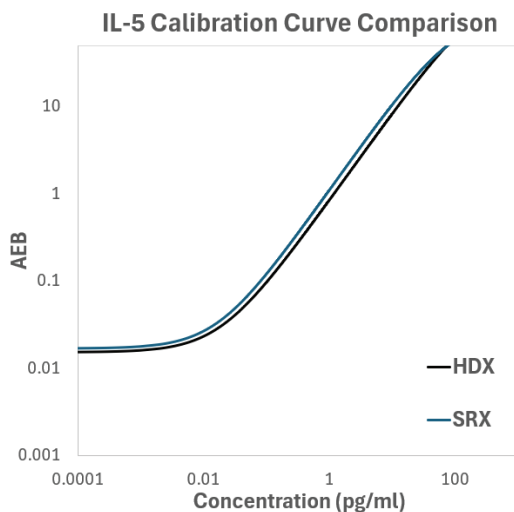


Figure 4. Comparison of the calibration curves for IL-5 in C4PB Advantage PLUS kits across HD-X (black) and SR-X (blue) platforms for one reagent kit lot.

## II. Sample Reading

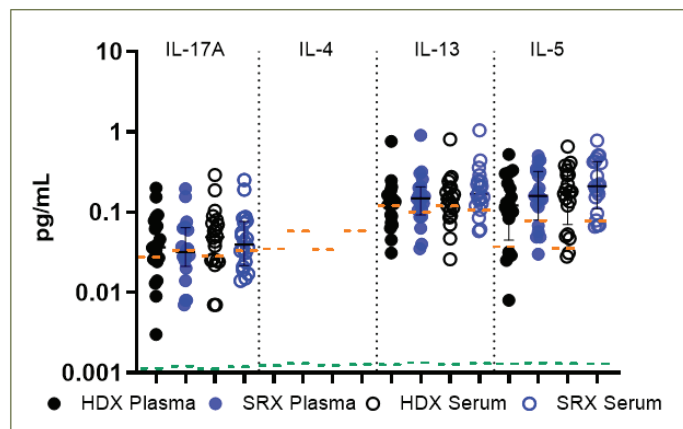


Figure 5. C4PB levels for 20 serum and 20 EDTA plasma matched samples from presumably healthy individual donors on HD-X and SR-X instruments for one reagent kit lot. The orange dashed lines indicate the functional LLOQ and green dashed line indicate the LOD of the C4PB Advantage PLUS assays.

### III. Sample Reading Comparison

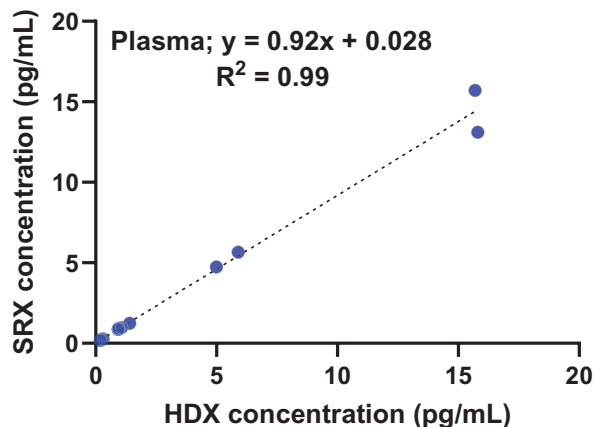
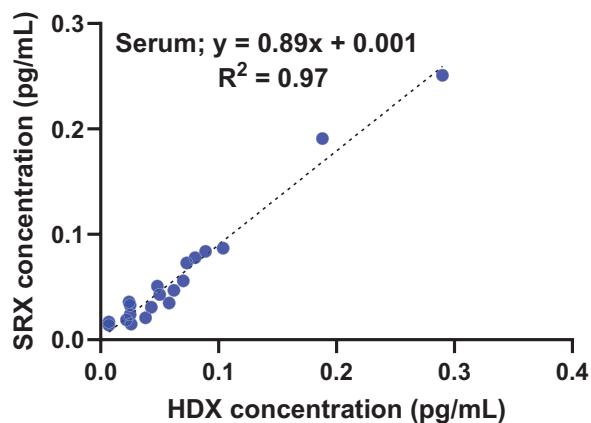
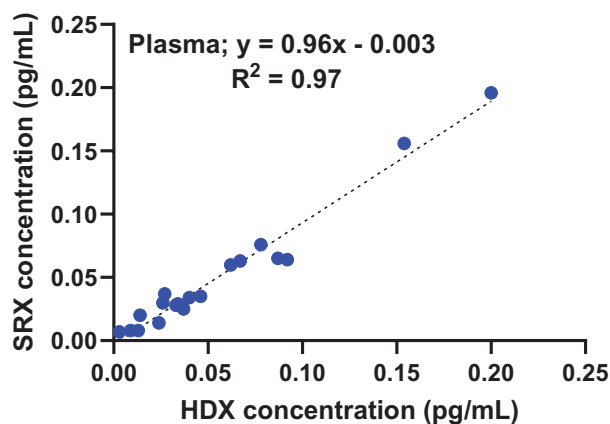


Figure 7. Correlation between HD-X and SR-X for IL-4 in C4PB Advantage PLUS assay for 16 antigen-spiked EDTA plasma (Slope 0.92,  $R^2$  0.99) samples for one reagent kit lot.

Figure 6. Correlation between HD-X and SR-X for IL-17A in C4PB Advantage PLUS assay for the 20 EDTA plasma (Slope 0.96,  $R^2$  0.97) and 20 serum (Slope 0.89,  $R^2$  0.97) matched samples for one reagent kit lot.

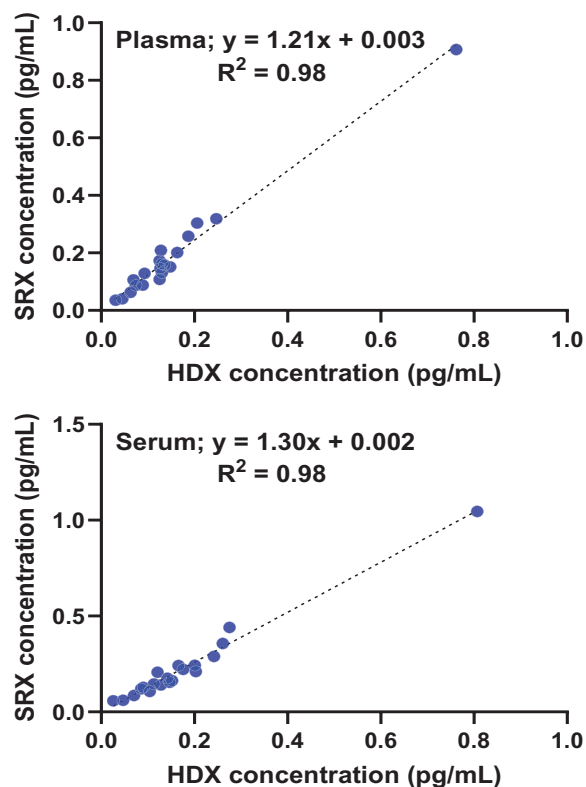


Figure 8. Correlation between HD-X and SR-X for IL-13 in C4PB Advantage PLUS assay for the 20 EDTA plasma (Slope 1.21,  $R^2$  0.98) and 20 serum (Slope 1.30,  $R^2$  0.98) matched samples for one reagent kit lot.

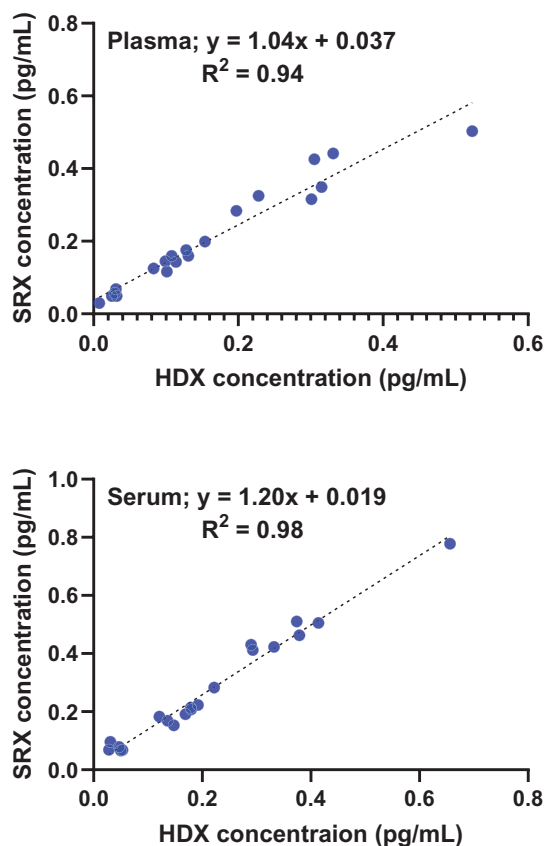


Figure 9. Correlation between HD-X and SR-X for IL-5 in C4PB Advantage PLUS assay for the 20 EDTA plasma (Slope 1.04,  $R^2$  0.94) and 20 serum (Slope 1.20,  $R^2$  0.98) matched samples from presumably healthy individual donors.

## Summary

Comparability of the C4PB Advantage PLUS (item #104997) between the Simoa HD-X Analyzer® and Quanterix SR-X® platforms was evaluated by measuring LLOQ, LOD, precision, detectability and quantifiability. The CAPB Advantage PLUS assay demonstrated similar sensitivity on the SR-X platform when compared to the HD-X platform, with minor variations in detectability and quantification limits observed across individual analytes.. The difference in sensitivity had a minor impact on sample detectability and quantifiability. C4PB detectability on the SR-X achieved 100% in both EDTA plasma and serum and quantifiability achieved 100% in both EDTA plasma and serum in IL-17A, IL-13, and IL-5. IL-4 of C4PB showed comparable detectability and quantifiability to HD-X, although a slope comparison indicates that, for some analytes, sample readout values may slightly differ across instruments. Overall, the C4PA Advantage PLUS assay was shown to function on the SR-X platform.

For more information on the C4PB Advantage PLUS assay and other Simoa® assay kits, please visit the Customer Portal: <https://portal.quanterix.com>

## References

1. Cytokine 4-plex B Advantage PLUS Validation Report
2. Cytokine 4-plex B Advantage PLUS Data Sheet