V-PLEX® Rat Proinflammatory Kit: Original (Panel 2) vs. New (Panel 1)

White Paper

Introduction

The V-PLEX Rat Proinflammatory Kit has been updated with improved antibodies and calibrator for increased sensitivity. In this white paper, we discuss the key performance metrics of the new V-PLEX Proinflammatory Panel 1 (rat) Kit and the original V-PLEX Proinflammatory Panel 2 (rat) Kit.

Table 1: Kits compared in this white paper.

Description	Part Number Original (Panel 2)	Part Number New (Panel 1)
V-PLEX Rat Proinflammatory Kit	K15059D/G	K15294D/G

Methods

The V-PLEX Proinflammatory Panel 1 (rat) Kit uses updated antibodies for 7 assays (IFN-γ, IL-1β, IL-4, IL-5, IL-6, IL-10, and KC/GRO) and adds an assay for IL-2 assay. Additionally, the panel uses a new assay diluent to reduce matrix effects, uses new antibody diluent and read buffer to improve performance, and eliminates a blocking step to reduce protocol time by 1 hour. These changes also lead to improved sensitivity and increased longevity of critical reagents.

Table 2: Differences in antibody generation and reagents between the original (Panel 2) and new (Panel 1) versions of the V-PLEX Rat Proinflammatory Kit.

Description	Original (Panel 2)	New (Panel 1)
Assay Generation:		
IFN-γ	A	B
IL-1β		
IL-4		
IL-5		
IL-6		
IL-10		
KC/GRO		
TNF-α	A	A
IL-2	Absent	Present
Blocking step	Yes (1 h)	No
Blocker	Blocker H	NA
Assay diluent	Diluent 42	Diluent 65 + EDTA
Antibody diluent	Diluent 40	Diluent 3
Read buffer	Read Buffer T (2X)	MSD GOLD™ Read Buffer B

A = Original assay
B = New assay

Calibration Curve Comparison

The calibrators in the V-PLEX Proinflammatory Panel 1 (rat) Kit are anchored to the calibrators in the V-PLEX Proinflammatory Panel 2 (rat) Kit to ensure consistent quantitation between kits. As shown in Figure 1, all but IL-6 calibration curves from the V-PLEX Proinflammatory Panel 1 (rat) Kit (red) have equivalent or higher sensitivity and range relative to the calibration curves from the V-PLEX Proinflammatory Panel 2 (rat) Kit (blue). The new IL-6 assay is included in the V-PLEX Proinflammatory Panel 1 (rat) Kit to ensure a more consistent assay performance with the updated diluents.

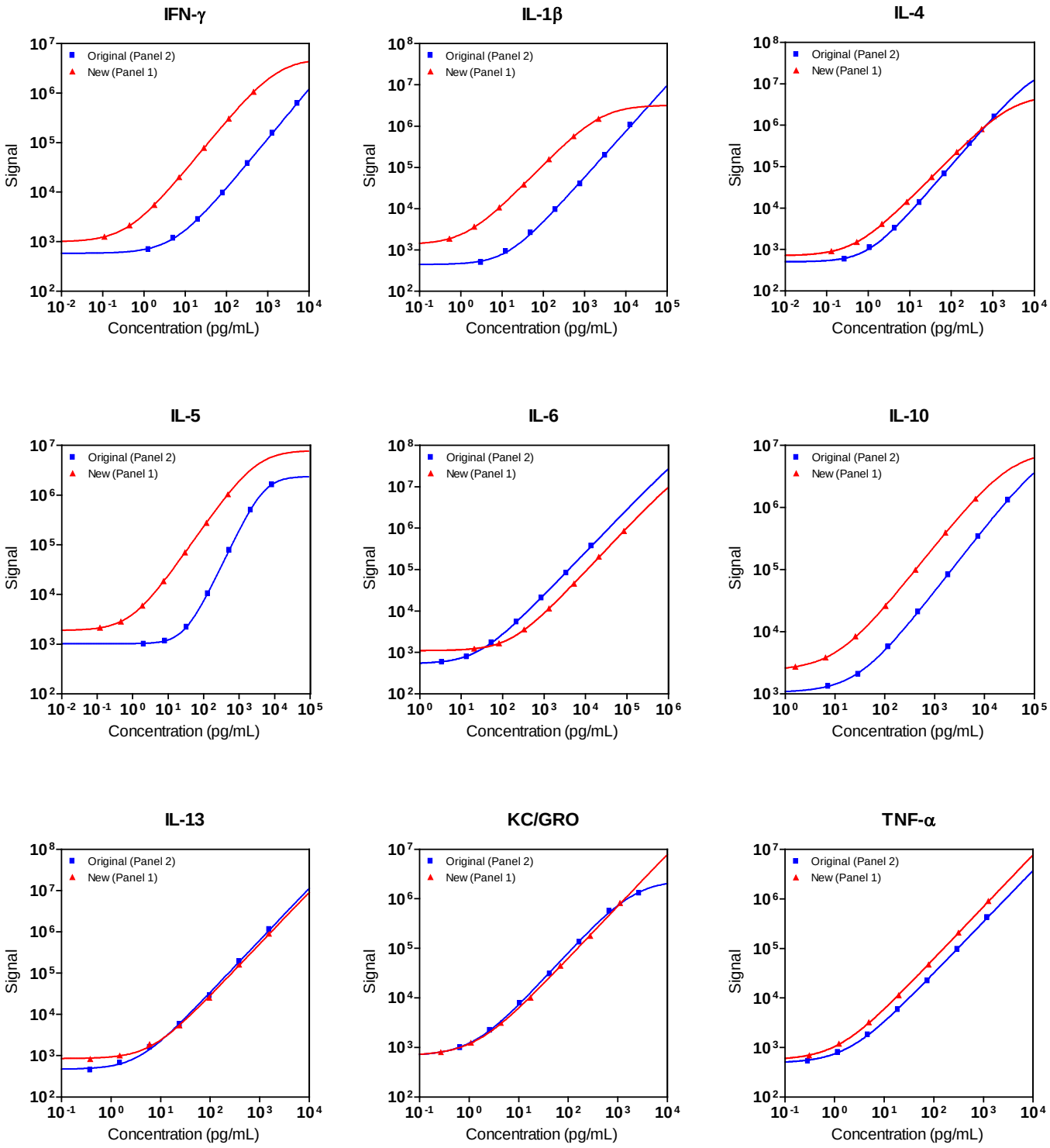

Figure 1: Comparison of calibration curves for the original (Panel 2) and new (Panel 1) versions of the V-PLEX Rat Proinflammatory Kit.

Sensitivity, Slope, and Dynamic Range Comparison

As shown in Table 3, nearly all assays in the new (Panel 1) kit have equivalent or improved sensitivity. Antibody changes significantly improved Hill slopes, LLODs, and LLOQs. Top-of-curve and ULOQ concentrations were adjusted to maximize dynamic range.

The lower limit of detection (LLOD) is a calculated concentration corresponding to 2.5 standard deviations above the background (zero calibrator). The upper limit of quantitation (ULOQ) is the highest concentration at which the CV of the calculated concentration is ≤25% and the recovery of each analyte is within 75-125% of the nominal value. The LLOQ is the lowest concentration at which the CV of the calculated concentration is ≤25% and the recovery of each analyte is within 75-125% of the nominal value.

Table 3: Comparison of the original (Panel 2) and new (Panel 1) versions of the V-PLEX Rat Proinflammatory Kit.

	Hill slope		Median LLOD (pg/mL)		LLOQ (pg/mL)		ULOQ (pg/mL)	
Analyte	Original (Panel 2)	New (Panel 1)	Original (Panel 2)	New (Panel 1)	Original (Panel 2)	New (Panel 1)	Original (Panel 2)	New (Panel 1)
IFN-γ	1.01	1.03	0.65	0.03	39.7	1.21	3,750	292
IL-1β	1.12	1.03	6.92	0.11	102	2.66	8,100	1,230
IL-4	1.16	1.02	0.69	0.08	8.00	3.62	723	357
IL-5	1.52	1.03	14.1	0.06	82.0	1.86	3,000	305
IL-6	1.04	1.06	13.8	13.06	96.9	188	8,550	54,600
IL-10	1.04	1.02	16.4	1.80	163	43.1	15,700	3,900
IL-13	1.27	1.26	1.97	0.90	12.5	11.8	1,080	910
KC/GRO	1.10	1.05	1.04	0.16	21.7	2.23	728	604
TNF-α	1.04	1.05	0.72	0.17	9.10	2.44	793	715

Sample Testing Comparison

Fifty rat samples were tested (commercially sourced serum, EDTA plasma, heparin plasma, citrate plasma, and urine) at 4-fold dilution on the new V-PLEX Proinflammatory Panel 1 (rat) Kit. Results are presented below (Table 4).

Table 4: Normal rat samples tested in the V-PLEX Proinflammatory Panel 1 (rat) Kit.

Sample Type	Statistic	IFN-γ	IL-1β	IL-4	IL-5	IL-6	KC/GRO	IL-10	IL-13	TNF-α
Serum (N=10)	Median (pg/mL)	4.59	ND	1.67	ND	ND	179	ND	9.28	5.24
	Range (pg/mL)	2.87–6.30	NA	1.44–1.87	NA	NA	121–234	NA	NA	2.8–8.74
	% Detected	20%	0%	40%	0%	0%	100%	0%	10%	100%
	% Quantified	0%	0%	0%	0%	0%	100%	0%	0%	0%
EDTA Plasma (N=10)	Median (pg/mL)	4.59	14.3	3.84	630	17.1	24.5	ND	6.27	8.13
	Range (pg/mL)	NA	NA	1.42–6.26	NA	NA	20.8–94	NA	3.53–7.00	5.26–20.6
	% Detected	10%	10%	20%	10%	10%	100%	0%	30%	100%
	% Quantified	0%	0%	0%	10%	0%	10%	0%	0%	0%
Heparin Plasma (N=10)	Median (pg/mL)	5.18	ND	2.10	ND	ND	78.9	ND	6.41	12.3
	Range (pg/mL)	3.33–8.84	NA	1.77–2.75	NA	NA	63.7–141	NA	3.07–10.1	5.76–14.8
	% Detected	60%	0%	60%	0%	0%	100%	0%	80%	100%
	% Quantified	0%	0%	0%	0%	0%	30%	0%	0%	0%
Citrate Plasma (N=10)	Median (pg/mL)	5.78	9.67	2.22	ND	ND	22.3	ND	4.7	10.3
	Range (pg/mL)	3.27–12.3	NA	1.76–2.91	NA	NA	12.8–36.5	NA	3.72–6.69	5.64–16.2
	% Detected	90%	10%	90%	0%	0%	100%	0%	80%	100%
	% Quantified	0%	0%	0%	0%	0%	0%	0%	0%	0%
Urine (N=10)	Median (pg/mL)	ND	ND	ND	ND	ND	21.5	ND	ND	3.75
	Range (pg/mL)	NA	NA	NA	NA	NA	1.54–60.4	NA	NA	2.23–6.57
	% Detected	0%	0%	0%	0%	0%	100%	0%	0%	40%
	% Quantified	0%	0%	0%	0%	0%	0%	0%	0%	0%

ND = Not detectable
NA = Not applicable

Concordance Comparison

The same samples were also tested on the V-PLEX Proinflammatory Panel 2 (rat) Kit to evaluate concordance between the original and new versions of the kit. Samples with in-well concentration outside the dynamic range were excluded from the analysis. Results are shown below (Figure 2). Assays show good agreement for many analytes (IL-4, KC/GRO, IL-13 and TNF-α). Certain analytes, such as: IL-1β, IL-5, IL-6 and IL-10, have low detectability, which was expected due to their naturally low abundance in the tested sample matrices. For IFN-γ, the new assay is significantly more sensitive than the original, allowing the detection of samples that the original cannot, leading to low concordance between the two assays.

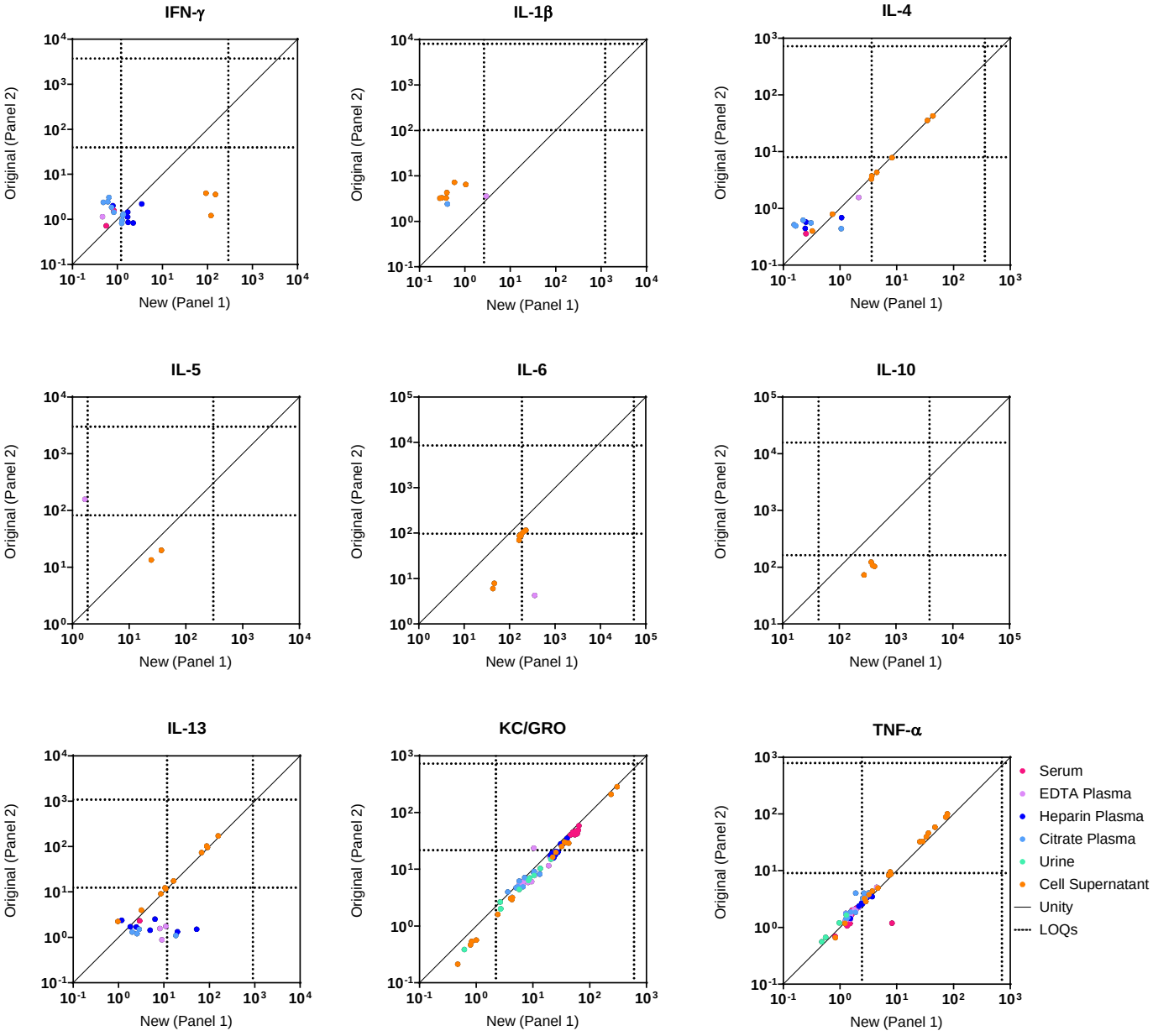

Figure 2: Rat sample concordance between the V-PLEX Proinflammatory Panel 1 (rat) Kit (x-axis) and the V-PLEX Proinflammatory Panel 2 (rat) Kit (y-axis).

Table 5: R-squared, slope and number of samples for each analyte in figure 2.

Assay	r^2	Slope	N
IFN-γ	0.265	0.011	21
IL-1β	0.003	25.3	8
IL-4	0.999	0.999	19
IL-5	n/a	-4.88	3
IL-6	0.050	0.138	9
KC/GRO	0.993	0.908	66
IL-10	0.520	0.258	4
IL-13	0.920	1.13	25
TNF-α	0.996	1.24	62

Summary

The V-PLEX Proinflammatory Panel 1 (rat) Kit is an analytically validated and improved version of the V-PLEX Proinflammatory Panel 2 (rat) Kit with updated antibodies as well as new assay and antibody diluents, read buffer, and protocol for improved sensitivity and increased longevity of critical reagents. The V-PLEX Proinflammatory Panel 1 (rat) Kit demonstrates reliable sample quantitation and strong concordance with the V-PLEX Proinflammatory Panel 2 (rat) Kit for multiple analytes. More information and data can be found in the product insert for each panel.

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