M1035

Human V-PLEX® Multiplex Kit Performance: Multi-Year Longitudinal Analysis

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1 Abstract

Purpose: In the field of immunoassay-based biomarker measurements, one of the most significant challenges is assuring consistent assay performance over time. This is especially difficult in multiplexed systems given the number and diversity of components that make up an assembled assay kit; each component requires careful characterization and qualification to ensure reproducible lot-to-lot kit performance. A retrospective analysis was conducted on the quality control results for more than 120 lots of MSD® human V-PLEX multiplex kits produced over the past four years. The review demonstrates that a high level of consistency and reproducibility can be achieved for biomarker measurements using analytically validated multiplex kits created under MSD's comprehensive development, verification, and validation processes.

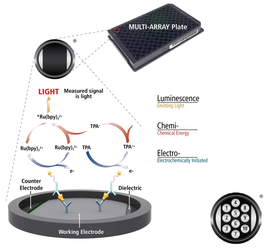
Methods: Quality control methods for releasing V-PLEX kits included measuring the precision (intra-plate and inter-plate %CVs) and accuracy (recovery of measured concentrations relative to expected values) for calibrators, lower and upper limit of quantitation (LLOQ and ULOQ) samples, and control samples. Each kit contained plates, calibrators, detection antibodies, and diluents, and the performance of each component was verified individually prior to being accepted for inclusion in a kit lot. Quality control samples used for kit testing were independently built and their concentrations were assigned across multiple kit lots. The quality control process for each kit lot included testing multiple plates with a minimum of four replicates per plate for each measurement.

Results: The inter-plate concentration %CV for each assay's mid-level calibrator was typically below 10%, with all measurements falling below 20%. The average intra-plate concentration %CV for each assay's LLOQ and ULOQ samples were below 20% and the measured recoveries were within 20% of the expected concentration. The precision and accuracy of high, mid, and low control levels were measured for each assay, and each control produced an intra-plate concentration %CV below 20% and a measured recovery within 20% of the expected concentration.

Conclusion: A multi-lot and multi-year analysis of human V-PLEX multiplex kit quality control testing showed consistency and reproducibility that validates the suitability of these products for long-term studies.

2 Methods

MSD's electrochemiluminescence detection technology uses SULFO-TAG™ labels that emit light upon electrochemical stimulation initiated at the electrode surfaces of MULTI-ARRAY® and MULTI-SPOT® microplates.



Electrochemiluminescence Technology

- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed assays.
- Labels are stable, non-radioactive, and directly conjugated to biological molecules.
- Emission at ~620 nm eliminates problems with color quenching.
- Multiple rounds of label excitation and emission enhance light levels and improve sensitivity.
- Carbon electrode surface has 10X greater binding capacity than polystyrene wells.
- Surface coatings can be customized.

Quality Control Methods

Quality control testing of V-PLEX kits included measuring the precision (intra-plate and inter-plate %CVs) and accuracy (recovery of measured concentrations relative to expected values) for calibrators, LLOQ (lower limit of quantitation and ULOQ (upper limit of quantitation) samples, and control samples.
Limit of detection is a calculated concentration corresponding to the average signal at 2.5 standard deviations above the background (zero calibrator). LLOQ and ULOQ are established for the plate lot by measuring multiple levels of calibrator near the expected LLOQ and ULOQ. LLOQ and ULOQ are the lowest and highest (respectively) concentrations of calibrator tested which have %CVs of 20% or less, with recovered concentrations within 70-130%.
High, Mid, and Low QC controls are prepared by spiking recombinant calibrators into a non-human serum matrix.
The protocol utilized during the release of MSD human V-PLEX multiplex kits is shown at right.

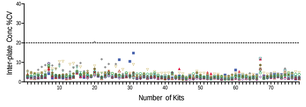
Protocol

1. Wash plate 3 times with 150 µL/well Wash Buffer. Add 50 µL/well of sample (calibrator, controls, LOQ samples). Incubate at room temperature with shaking for 2 hours.
2. Wash plate 3 times with 150 µL/well Wash Buffer. Add 25 µL/well of 1X detection antibody solution. Incubate at room temperature with shaking for 2 hours.
3. Wash plate 3 times with 150 µL/well Wash Buffer. Add 150 µL/well of 2X Read Buffer T. Read on the MSD instrument.

3 Proinflammatory Panel 1 (human)

The following figures illustrate the quality control test results for 78 independently tested Proinflammatory Panel 1 (human) Kit lots. Testing was performed between May 2013 and September 2016 using 6 different control lots. Each data point represents the results compiled for a minimum of 5 plates.

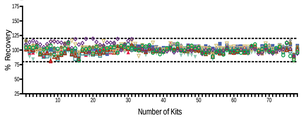
1 Inter-plate concentration %CV measurements for a mid-level calibrator sample across 78 kits



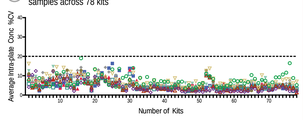
Assay Legend for Plots 1-6

hIFN-γ	hIL-8
hIL-1β	hIL-10
hIL-2	hIL-12p70
hIL-4	hIL-13
hIL-6	hTNF-α

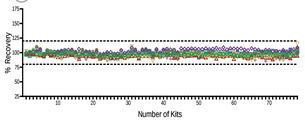
2 % Recovery measurements for LLOQ samples across 78 kits



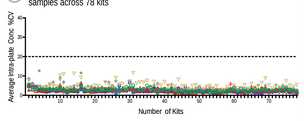
3 Average intra-plate concentration %CV measurements for LLOQ samples across 78 kits



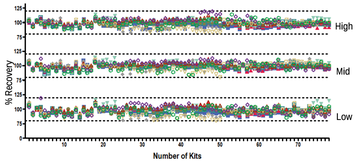
4 % Recovery measurements for ULOQ samples across 78 kits



5 Average intra-plate concentration %CV measurements for ULOQ samples across 78 kits



6 % Recovery measurements for High, Mid, and Low Control samples across 78 kits are plotted below.
Average % recovery and intra-plate concentration %CVs are shown in the table to the right.

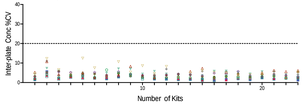


	Average % Recovery			Average Intra-plate Conc. %CV		
	High	Mid	Low	High	Mid	Low
hIFN-γ	94.9	97.9	93.9	3.1	2.1	2.1
hIL-1β	99.4	99.8	98.6	3.2	2.5	3.1
hIL-2	100.3	100.1	99.6	3.0	2.6	3.0
hIL-4	97.6	100.2	94.4	3.3	3.2	3.2
hIL-6	97.6	98.7	96.9	2.5	2.4	2.4
hIL-8	98.7	98.2	97.9	2.4	2.4	2.5
hIL-10	101.8	101.0	101.3	1.9	1.7	2.3
hIL-12p70	94.8	97.2	92.9	4.7	4.5	5.0
hIL-13	102.2	101.4	99.3	2.2	2.3	2.4
hTNF-α	99.6	96.9	96.7	2.8	2.7	4.0

4 Chemokine Panel 1 (human)

The following figures illustrate the quality control test results for 23 independently tested Chemokine Panel 1 (human) Kit lots. Testing was performed between July 2013 and January 2017 using 3 different control lots. Each data point represents the results compiled for a minimum of 5 plates.

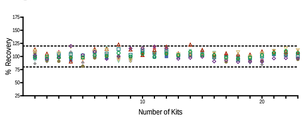
7 Inter-plate concentration %CV measurements for a mid-level calibrator sample across 23 kits



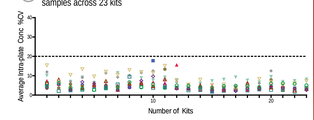
Assay Legend for Plots 7-12

hEotaxin	hMIP-1α
hMIP-1β	hIL-8
hEotaxin-3	hMCP-1
hTARC	hMDC
hIP-10	hMCP-4

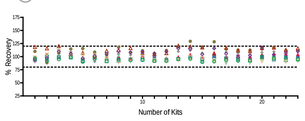
8 % Recovery measurements for LLOQ samples across 23 kits



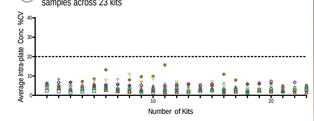
9 Average intra-plate concentration %CV measurements for LLOQ samples across 23 kits



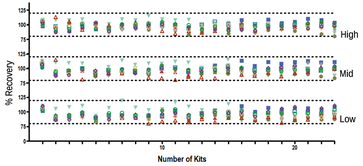
10 % Recovery measurements for ULOQ samples across 23 kits



11 Average intra-plate concentration %CV measurements for ULOQ samples across 23 kits



12 % Recovery measurements for High, Mid, and Low Control samples across 23 kits are plotted below.
Average % recovery and intra-plate concentration %CVs are shown in the table to the right.

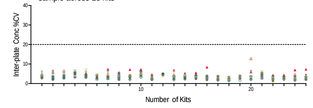


	Average % Recovery			Average Intra-plate Conc. %CV		
	High	Mid	Low	High	Mid	Low
hEotaxin	99.0	101.9	98.4	2.2	1.7	2.4
hMIP-1β	97.1	96.1	93.0	2.6	2.1	2.3
hEotaxin-3	102.2	101.4	100.4	3.8	3.5	4.4
hTARC	93.4	97.6	92.6	3.8	3.0	3.4
hIP-10	93.3	93.3	91.9	5.2	3.8	3.7
hMIP-1α	99.2	98.3	96.2	1.6	1.5	2.7
hIL-8	97.5	91.6	87.3	2.6	2.1	2.5
hMCP-1	96.0	96.6	92.3	4.7	4.3	5.0
hMDC	95.4	97.8	97.5	4.2	3.6	3.2
hMCP-4	98.2	97.9	97.5	2.0	2.1	2.9

5 Cytokine Panel 1 (human)

The following figures illustrate the quality control test results for 25 independently tested Cytokine Panel 1 (human) Kit lots. Testing was performed between May 2013 and March 2017 using 5 different control lots. Each data point represents the results compiled for a minimum of 5 plates.

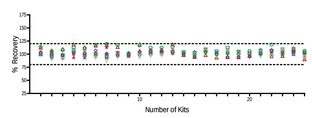
13 Inter-plate concentration %CV measurements for a mid-level calibrator sample across 25 kits



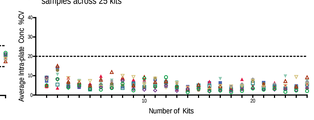
Assay Legend for Plots 13-18

hGM-CSF	hIL-15
hIL-1α	hIL-16
hIL-5	hIL-17A
hIL-7	hTNF-β
hIL-12p40	hVEGF-A

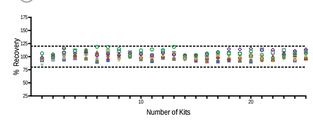
14 % Recovery measurements for LLOQ samples across 25 kits



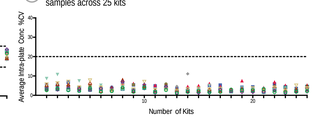
15 Average intra-plate concentration %CV measurements for LLOQ samples across 25 kits



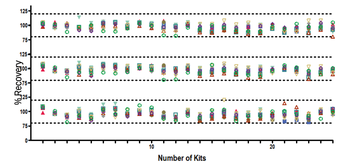
16 % Recovery measurements for ULOQ samples across 25 kits



17 Average intra-plate concentration %CV measurements for ULOQ samples across 25 kits



18 % Recovery measurements for High, Mid, and Low Control samples across 25 kits are plotted below.
Average % recovery and intra-plate concentration %CVs are shown in the table to the right.



	Average % Recovery			Average Intra-plate Conc. %CV		
	High	Mid	Low	High	Mid	Low
hGM-CSF	97.8	99.7	93.9	3.8	2.7	3.1
hIL-1α	100.1	99.7	97.1	4.8	4.1	5.1
hIL-5	100.8	101.4	99.1	3.3	3.1	3.3
hIL-7	96.7	98.5	95.4	3.3	2.8	3.2
hIL-12p40	96.2	97.8	93.3	2.7	2.5	2.8
hIL-15	95.4	97.3	96.7	3.6	2.7	3.1
hIL-16	93.6	94.4	95.0	3.3	3.1	3.6
hIL-17A	98.4	99.7	96.7	3.2	3.0	3.2
hTNF-β	98.4	97.7	94.7	2.4	2.5	2.7
hVEGF-A	95.4	94.7	95.9	2.0	2.1	2.4

6 Conclusion

A retrospective analysis of MSD's rigorous quality control testing on more than 120 lots of human V-PLEX multiplex kits demonstrated high levels of precision and accuracy. Inter-plate concentration CVs were typically below 10%, with average measurements for intra-plate concentration CVs below 20% and measured recoveries within 20% of the expected concentration. The analysis confirmed the consistency and reproducibility of V-PLEX assay performance, demonstrating the suitability of these products for longitudinal studies.

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