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Development and Characterization of V-PLEX® TH17 Cytokine Assays

Victoria Yatsula, Lori Kobayashi, Deepak Sharma, Elisa Qualters, Lalitha Janaki, Celine Weckerle, Seth B. Harkins, David Stewart, and Jacob N. Wohlstadter

Meso Scale Discovery, Rockville, Maryland, USA

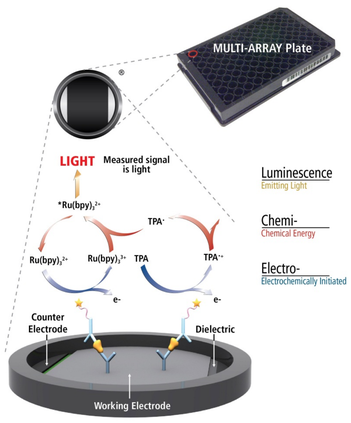
1 Introduction

Th17 cytokines are important mediators of the host defense against infection and are increasingly investigated for their role in autoimmune disorders and immune regulation at mucosal surfaces. We described the development, characterization, and analytical validation of a multiplexed immunoassay panel for seven Th17 cytokines, IL-17A, IL 21, IL-22, IL-23, IL-27, IL-31, and MIP-3alpha, on MSD's V-PLEX platform.

To efficiently and rapidly identify potential antibody pairs, biotinylated capture antibodies and detection antibodies conjugated with SULFO-TAG™ label were screened on MSD's U-PLEX® platform, which enables the solution phase assembly of capture antibody arrays. Subsequent development used printed arrays of capture antibodies. Antibody concentrations, calibrator curve linearity, dynamic range, specificity, matrix tolerance, and assay robustness were analyzed for each assay during development.

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.

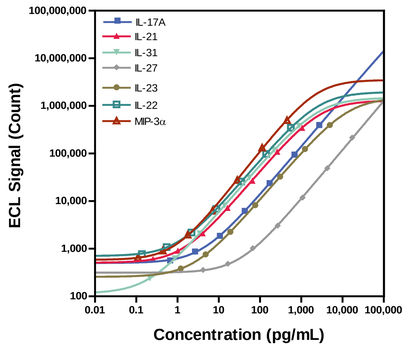


Electrochemiluminescence Technology

- 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.

3 Calibration Curves and Limits of Detection

The figure below demonstrates typical calibration curves for the analytes in the TH17 Panel 1 (human) Kit. The table below demonstrates typical data for the seven assay in the V-PLEX TH17 Panel 1 Kit. Data include Hill slopes, lower limit of quantification (LLOQ) values, and ranges for the lower limits of detection (LLOD, n=60 runs). The calibrator was reconstituted and diluted serially (4-fold) to generate a 7-point standard curve.



Spot 1: IL-17A
Spot 2: BSA
Spot 3: BSA
Spot 4: IL-21
Spot 5: IL-31
Spot 6: IL-27
Spot 7: IL-23
Spot 8: IL-22
Spot 9: BSA
Spot 10: MIP-3α

Calibration Curve Parameters							
	IL-17A	IL-21	IL-22	IL-23	IL-27	IL-31	MIP-3α
Hill Slope	1.02	1.03	1.01	1.01	1.04	1.0	1.12
LLOQ (pg/mL)	5.86	1.65	2.78	4.60	38.7	4.22	0.588
LLOD Range (pg/mL)	0.148–2.15	0.059–1.20	0.040–1.99	0.041–1.99	1.86–29.0	0.218–1.08	0.020–0.270

4 Specificity

Analytes from the V-PLEX TH17 Panel 1 Kit were assessed for specificity with recombinant analytes from other human V-PLEX kits including: Proinflammatory Panel 1, Cytokine Panel 1, Chemokine Panel 1, Angiogenesis Panel 1, and Vascular Injury Panel 2.

Calibrator blends were tested for 48 analytes: bFGF, CRP, Eotaxin, Eotaxin-3, Flt-1, G-CSF, GM-CSF, ICAM-1, IFN-γ, IL-10, IL-12/23p40, IL-12p70, IL-13, IL-15, IL-16, IL-17A, IL-17A/F, IL-17B, IL-17C, IL-17D, IL-1RA, IL-1α, IL-1β, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IP-10, MCP-1, MCP-4, MDC, MIP-1α, MIP-1β, PIGF, SAA, TARC, Tie-2, TNF-α, TNF-β, TSLP, VCAM-1, VEGF, VEGF-C, VEGF-D.

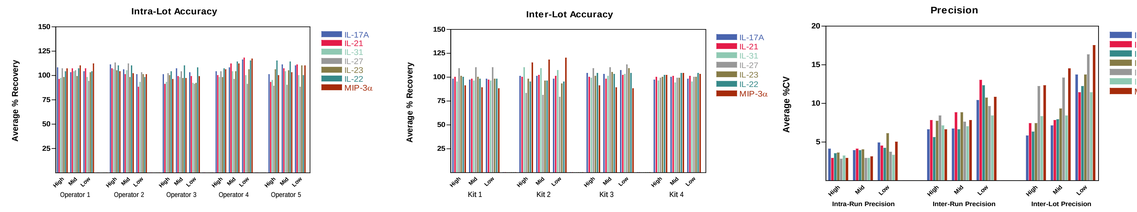
Non-specific binding was less than 0.5% for all assays.

IL-17A assay showed 9.4% cross-reactivity against IL-17A/F calibrator.

5 Accuracy and Precision

Quality control samples were made by spiking calibrator into assay diluent at three levels (high, mid, and low) within the quantitative linear range of the assay. The controls were measured using a minimum of three replicates tested over multiple days and multiple operators for a total of 60 runs.

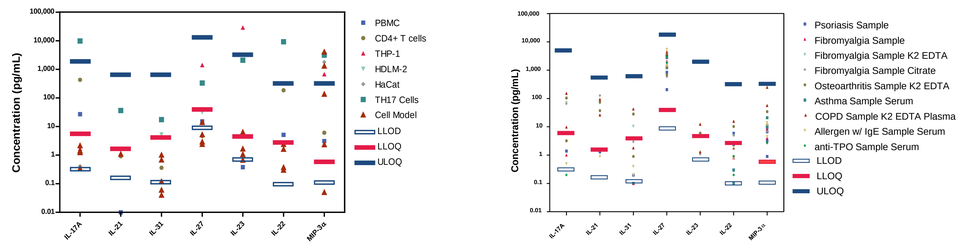
- Intra-lot accuracy was defined as the average measured control concentration for a given lot divided by the expected control concentration assigned from previous runs. The intra-lot accuracy (n=3 runs) is shown for five different operators.
- Inter-lot accuracy was defined as the average measured control concentration for different lots divided by the expected control concentration. The inter-lot accuracy (n=15 runs) is shown for four different kits.
- Intra-run precision was defined as the average %CV of the control replicates (n=3) within an individual plate averaged across 12 plates.
- Inter-run precision was defined as the %CV from control concentrations measured on a kit (n=15).
- Inter-lot precision was reported as the %CV from control concentrations measured across four kit lots.



The accuracy of control determinations was within 20% of expected concentration with precision of less than 20% CV.

6 Stimulated and Disease Sample Testing

Stimulated and diseased samples in a variety of matrices were purchased and tested against analytes on the TH17 Panel 1 (human) Kit. Most analytes were detectable within the LLOQ and ULOQ ranges thus confirming the sensitivity and utility of the panel. Notably, all analytes were detectable in the Th17 cell model.



7 Dilution Linearity

Serum and plasma samples from a commercial source (n=10) and cell culture media (n=6) were diluted 2-fold to 32-fold. The average % recovery fell within the 80-120% range.

Dilution Linearity Average % Recovery								
Sample Type	Fold Dilution	IL-17A	IL-21	IL-22	IL-23	IL-27	IL-31	MIP-3α
Serum (n=10)	4	100	100	100	100	100	100	100
	8	104	92	104	105	104	100	104
	16	105	94	107	111	104	104	107
	32	104	94	103	133	115	98	125
EDTA Plasma (n=10)	4	100	100	100	100	100	100	100
	8	96	99	101	91	98	89	99
	16	96	114	103	90	104	94	93
	32	97	127	103	103	110	96	100
Cell Culture Supernatant (n=6)	4	100	100	100	100	100	100	100
	8	94	94	94	96	101	96	98
	16	98	97	93	89	103	99	101
	32	97	98	102	96	116	102	117

8 Conclusion

Calibration curves demonstrated a three-log dynamic range while achieving a LLOQ of less than 6 pg/mL for the majority of the assays. Dilution linearity and spike recovery studies in serum, plasma, urine (data not shown), and cell culture media were conducted to demonstrate matrix compatibility. Cross-reactivity was shown to be less than 0.3% between assays within the panel and less than 0.5% when panned against more than 30 other blood-related cytokines and biomarkers. These validated multiplexed assays provided sensitive measurement of Th17 cytokines in a variety of matrices and can be used as part of a researcher's studies.

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