M1038

Development and Analytical Validation of V-PLEX® TH17 Cytokine Assays

Lori Kobayashi, Lalitha Janaki, Jennifer Morgan, Elisa Qualters, Deepak Sharma, Seth B. Harkins, David Stewart, Pankaj Oberoi, and Jacob N. Wohlstadter

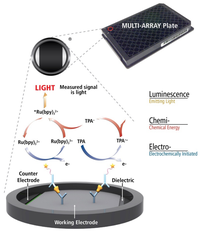
Meso Scale Discovery, Rockville, Maryland, USA

1 Introduction

Th17 cytokines are important mediators of the host defense against infection and are increasingly being investigated for their role in autoimmune disorders and immune regulation at mucosal surfaces. Here we describe the development, characterization, and analytical validation of a multiplexed immunoassay panel for Th17 cytokines on MSD's validated V-PLEX platform. Cytokines included in the panel were human IL-17A, IL-21, IL-22, IL-23, IL-27, IL-31, and MIP-3alpha. To provide for efficient and high-throughput screening of potential antibodies, 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 multiplex capture antibody arrays. Subsequent development used printed arrays of capture antibodies. Development of individual assays took antibody concentrations, calibrator curve linearity, dynamic range, specificity, matrix tolerance, and assay robustness into consideration. In the final assay configuration, numerous controls and samples were simultaneously tested for all analytes in the panel across multiple kits lots produced during development and validation.

2 Methods

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

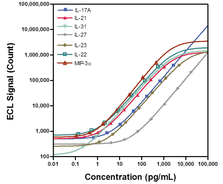


Electrochemiluminescence Technology

- 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

The following figure demonstrates typical calibration curves for the analytes in the TH17 Panel 1 (human) Kit.



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

4 Limits of Detection

The following table demonstrates typical data for the seven assays 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 is reconstituted and diluted serially (4-fold) to generate a 7-point standard curve.

	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

5 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 the 48 analytes shown in the table below:

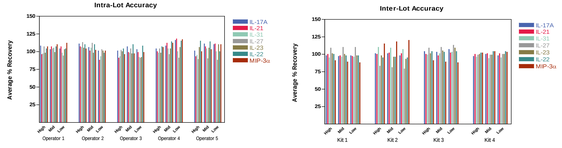
bFGF	IFN-γ	IL-17A/F	IL-3	MCP-1	Tie-2
CRP	IL-10	IL-17B	IL-4	MCP-4	TNF-α
Eotaxin	IL-12/23p40	IL-17C	IL-5	MDC	TNF-β
Eotaxin-3	IL-12p70	IL-17D	IL-6	MIP-1α	TSLP
Flt-1	IL-13	IL-1RA	IL-7	MIP-1β	VCAM-1
G-CSF	IL-15	IL-1α	IL-8	PlGF	VEGF-A
GM-CSF	IL-16	IL-1β	IL-9	SAA	VEGF-C
ICAM-1	IL-17A	IL-2	IP-10	TARC	VEGF-D

Non-specific binding was less than 0.5% for all assays. IL-17A assay showed 9.4% cross-reactivity against IL-17A/F calibrator.

6 Accuracy

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.

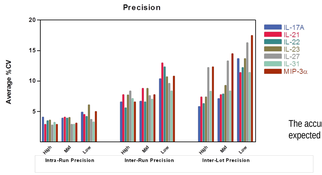
- Intra-lot accuracy is defined as the average measured control concentration for a given lot divided by the expected control concentration assigned from previous runs. The intra-lot accuracy (n=3 runs) is shown for five different operators.
- Inter-lot accuracy is defined as the average measured control concentration for different lots divided by the expected control concentration. The inter-lot accuracy (n=15 runs) is shown for four different kits.



7 Precision

High, mid, and low control samples prepared by spiking calibrator into assay diluent at three levels within the quantitative linear range were also used to assess precision. The controls were measured using a minimum of three replicates tested over multiple days and multiple operators for a total of 60 runs.

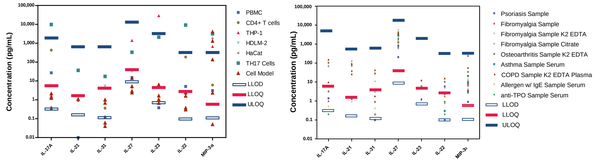
- Intra-run precision is defined as the average %CV of the control replicates (n=3) within an individual plate averaged across 12 plates.
- Inter-run precision is defined as the %CV from control concentrations measured on a kit (n=15).
- Inter-lot precision is reported as the %CV from control concentrations measured across four kit lots.



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

8 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 are 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.



9 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

10 Spike Recovery

Spike recovery measurements were evaluated for different sample types throughout the quantitative range of the assays. Multiple individual human samples (serum, EDTA plasma) were obtained from a commercial source. These samples, along with several different cell culture media, were spiked with calibrators at three levels (high, mid, and low) then diluted 2-fold. The average % recovery for each sample type is reported along with %CV and % recovery range.

	Serum (n=10)			EDTA Plasma (n=10)			Cell Culture Media (n=6)		
	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range	Average % Recovery	%CV	% Recovery Range
IL-17A	86	6.0	78 - 92	91	10.7	79 - 111	97	3.7	94 - 103
IL-21	101	10.5	85 - 115	38	53.6	15 - 82	105	3.7	100 - 110
IL-22	89	9.1	76 - 104	107	6.4	94 - 119	95	4.0	88 - 98
IL-23	44	38.1	19 - 72	90	7.9	77 - 101	96	3.8	92 - 102
IL-27	74	28.2	47 - 120	90	9.4	75 - 102	95	4.5	90 - 99
IL-31	84	9.3	70 - 93	91	15.2	64 - 112	101	3.3	97 - 105
MIP-3α	71	18.8	43 - 88	76	22.6	34 - 92	85	3.5	82 - 89

11 Conclusion

Calibration curves demonstrated a three-log dynamic range while achieving a lower limit of quantitation (LLOQ) of less than 1 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. Spiked matrix samples were typically found to recover between 80%-120% of the expected value. 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 provide sensitive measurement of Th17 cytokines in a variety of matrices and can be used as part of a researcher's studies.

Meso Scale Discovery
A division of Meso Scale Diagnostics, LLC.
www.mesoscale.com®

DOWNLOAD POSTER

MSD products are for research use only. Not for use in diagnostic procedures.

185-CY-417