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Development and Characterization of Th17 Related U-PLEX® Assays

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1 Abstract

Th17-related cytokines mediate a host's defense mechanisms against various infections and play a crucial role in crosstalk between the immune system and affected tissues. Here we describe the development and characterization of multiplexed Th17-related immunoassays on MSD's flexible U-PLEX platform.

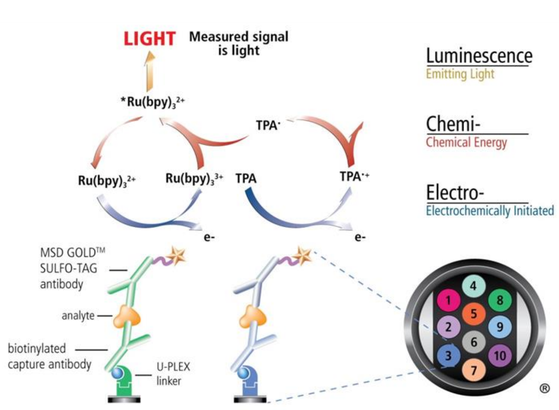
Monoclonal and polyclonal antibodies were conjugated with biotin and/or SULFO-TAG™ and screened as capture and/or detection reagents. A number of analytical parameters were used to select antibody pairs, and assays were developed by optimizing antibody concentrations, calibrator curves, specificity, matrix tolerance, and assay robustness. Assay performance was verified to be compatible with other U-PLEX assays by running Th17-related multiplexes on the U-PLEX platform with controls and samples.

Calibration curves showed expected signals, sensitivity, precision, and accuracy. Control samples for the assays had CVs < 10% within runs and < 25% between runs. Sensitivities were < 1 pg/mL for the majority of the assays. Dilution linearity and spike recovery studies demonstrated acceptable matrix tolerance and accurate quantification for most of the assays tested across all matrices (typically between 75% - 125%). Cross-reactivity between assays was shown to be typically < 0.5%. Results demonstrated a strong correlation between samples measured on U-PLEX multiplex and streptavidin plates with r^2 values > 0.95 and slopes between 0.8-1.2.

Over 20 Th17-related assays for human and mouse were developed for the U-PLEX platform. These assays can make biological measurements on matrices that are relevant to a wide range of life science applications and used as part of customer's pre-clinical or clinical studies.

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. The U-PLEX assay platform utilizes 10 unique linkers that specifically bind to individual spots enabling simple and flexible creation of multiplex immunoassays.

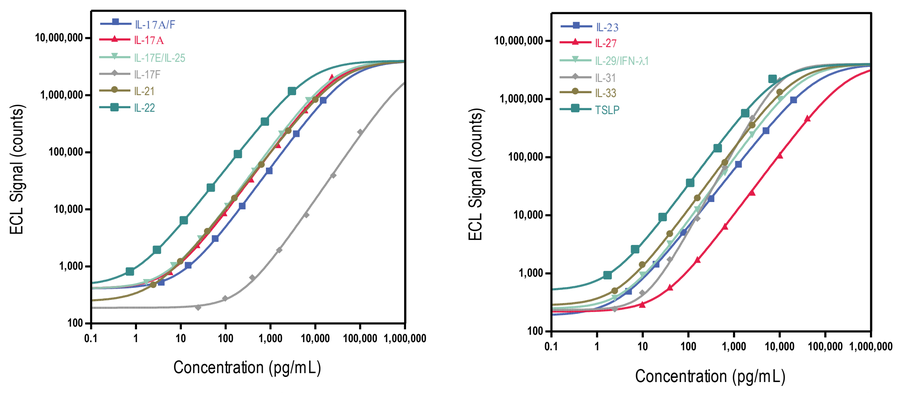


Electrochemiluminescence Technology

- 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 following figures demonstrate the typical calibration curves for 12 Th17 related (human) assays. Up to ten markers can be combined per plate to create the desired multiplex.



The following table demonstrates typical data for the 12 assays tested. Data include Hill slopes and lower limits of detection (LLOD) for the calibration curves. The calibrator is reconstituted and diluted serially (4-fold) to generate a 7-point standard curve.

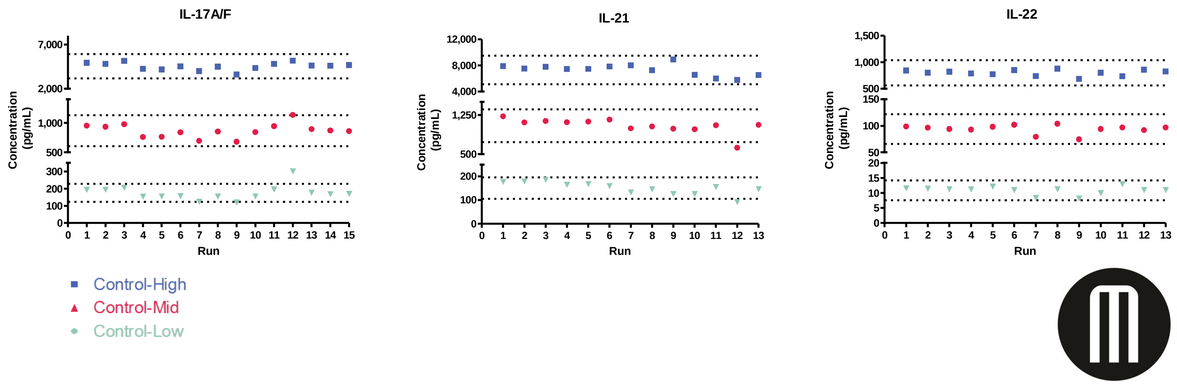
Average Hill slope and LLOD												
Assay	IL-17A/F	IL-17E / IL-25	IL-17F	IL-21	IL-22	IL-23	IL-27	IL-29 / IFN-λ1	IL-31	IL-33	TSLP	IL-17A
Hill slope	1.04	0.99	1.12	1.00	0.99	0.99	1.04	1.05	1.37	1.03	1.00	1.00
LLOD (pg/mL)	2.24	0.53	118	0.83	0.16	1.16	11.5	1.63	15.6	0.82	0.29	0.89

4 Specificity

The specificity for each of the markers was evaluated by testing for cross-reactivity for each capture–detector pair with all 51 analytes included in U-PLEX Biomarker Group 1 (human). No significant cross-reactivity (<0.5%) was observed within the 51 human analytes from the group.

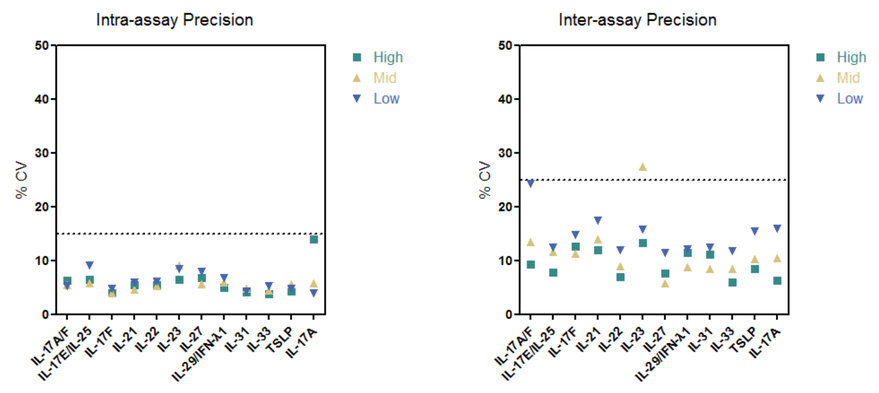
5 Reproducibility

Reproducibility of assays was evaluated by testing controls at three levels across the linear range of the calibration curve. The measured concentrations for three representative analytes are plotted in the graphs below. Assay performance is reproducible with almost all runs falling within the target range (70-130% of average).



6 Precision

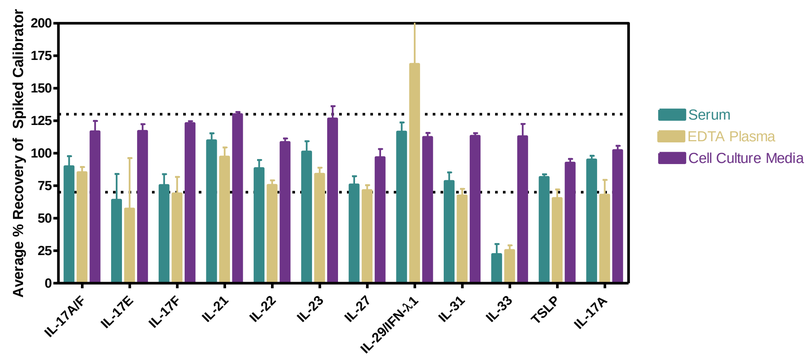
Controls were made by spiking calibrator into assay diluent at three levels (high, mid, and low) within the quantitative linear range of the assay. The controls were measured using a minimum of two replicates tested over multiple days on 14-20 runs (e.g., for IL-17A, n=6). Intra-assay precision is reported as the average %CV of the control replicates within an individual run. Inter-assay precision is reported as the %CV from controls measured across multiple runs.



7 Spike Recovery

Normal human serum (n=5) and EDTA plasma (n=5) samples from a commercial source and cell culture media were spiked with calibrators at three levels (high, mid, and low).

% Recovery = (measured concentration – sample endogenous level) / spiked concentration x 100



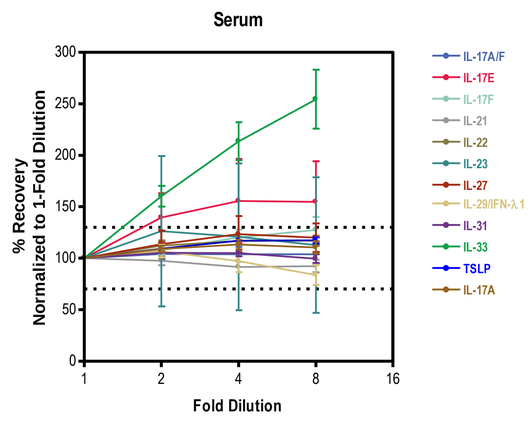
The percent recovery for most of the assays was within the targeted range (70–130%). IL-33 low recovery was due to high levels of receptor ST2 present in human serum and plasma. IL-29 recovery was found to be sample dependent with low recovery measured on a different set of samples (data not shown). Additional sample dilution may be used to reduce any matrix effect.

8 Dilution Linearity

To assess linearity, normal human serum (n=5) and EDTA plasma samples (n=5) from a commercial source, and cell culture media (n=5) were spiked with recombinant calibrator and diluted 1, 2, 4, and 8-fold before testing. The average percent recovery is based on samples that measured within the quantitative range of the assay.

% Recovery = (measured concentration / expected concentration) x 100

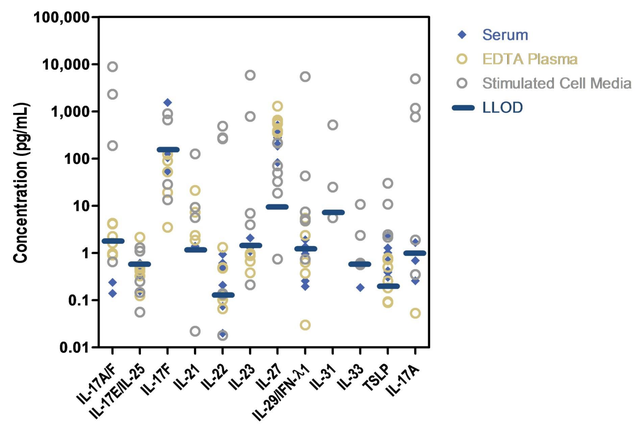
The percent recovery for serum samples is shown at right. Both serum and plasma samples were found to be within the targeted range (70–130%) for most of assays, suggesting that the samples dilute linearly from 1–8 fold. IL–33 and IL-17E were non-linear for both serum and plasma matrices, which may be associated with known receptor interferences and inter-sample variation.



9 Native Sample Testing

Normal human serum (n=8) and EDTA plasma (n=8) samples from a commercial source were tested neat. To demonstrate the detection of native analyte(s) in matrix, cell culture samples (n=6) that were stimulated with different compounds *in vitro* were also tested. Results for each sample set are displayed at right.

All the native analytes are detectable in the stimulated samples; many analytes are detectable in the normal serum and EDTA plasma as well.



10 Conclusion

The U-PLEX platform enables the rapid design and building of multiplex biomarker panels on the sensitive MSD® platform in a few easy steps. Optimization and characterization data from 12 Th17 related human biomarkers demonstrated sensitivity, precision and accuracy within and between assays.

More broadly, 114 biomarker assays (human: 51; mouse: 27; NHP: 36) have been successfully developed on the U-PLEX platform. Investigators can now combine novel biomarker assays using their own antibodies together with reagents and assays included in the U-PLEX menu. The antibody sets and calibrators provided by MSD have been optimized for performance and ease of use on the U-PLEX platform.

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