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Serum and Plasma IL-17A Concentrations in Lung Cancer Patients and Controls

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1 Abstract

Purpose: IL-17A, a cytokine produced by Th17 cells, is reportedly a central regulator of lung tumor growth (Reppert et al., Oncolmmunology, 2012) and a potential serum biomarker for lung cancer (Xu et al., Biomarkers, 2014). Serum concentrations of IL-17A are too low to measure in most samples (on the order of 0.1 pg/mL) using commercially available ELISAs. In order to overcome this limitation, we developed the S-PLEX™ Human IL-17A ultrasensitive assay which is capable of accurately measuring IL-17A in serum and plasma. We used this assay to measure IL-17A concentrations in serum samples from lung cancer patients and controls.

Methods: Serum samples from 59 lung cancer patients and 44 apparently healthy controls (including 14 heavy smokers) were analyzed using S-PLEX technology to determine IL-17A concentrations. In addition, IL-17A concentrations were measured in matched plasma samples from 24 of the controls. S-PLEX is a new ultrasensitive immunoassay format based on MSD's MULTI-ARRAY® electrochemiluminescence technology. The IL-17A assay requires only 25 µL of serum or plasma per measurement and can be run on the MESO® SECTOR S 600 and MESO QuickPlex® SQ 120 instruments.

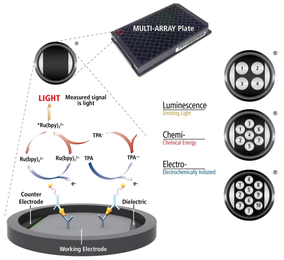
Results: The S-PLEX Human IL-17A assay was capable of accurately measuring IL-17A concentrations in 125 of the 127 samples tested. The lower limit of detection was 6 fg/mL and the assay range (LLOQ to ULOQ) was from 20 fg/mL to 200,000 fg/mL. Typical intra-plate coefficients of variation (CVs) ranged between 5% and 15%. Control samples run at 3 levels, in multiple replicates over multiple days, produced CVs <20%. Spike recovery and dilution linearity were between 80% and 120%, and IL-17A levels in matched serum and plasma samples were similar.

Specificity of the assay was demonstrated by analyte depletion using several anti-IL-17A specific antibodies, indicating the assay is specific for the IL-17A homodimer. There was no detectable cross-reactivity to the IL-17F homodimer, and the IL-17A/F heterodimer cross-reactivity was less than 1%. In this study, IL-17A serum concentrations were not found to be significantly different between lung cancer patients and controls, with a median concentration of 183 fg/mL and interquartile range (IQR) of 88-369 fg/mL (n=59) for lung cancer patients, compared to 128 fg/mL with an IQR of 75-198 fg/mL (n=44) for controls.

Conclusion: We have developed a highly specific and sensitive IL-17A assay that is 100 times more sensitive than the current limits of ELISA technology. This assay enables accurate determination of IL-17A concentrations in serum and plasma samples from lung cancer patients and healthy controls. The results from this study do not support the use of IL-17A as an effective serum biomarker for the presence of lung cancer.

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. We developed the S-PLEX assay platform, a next-generation MULTI-ARRAY technology with significantly higher sensitivity than traditional ELISAs. S-PLEX assays do not require specialized equipment and can be run on the MESO QuickPlex SQ 120, and all MESO SECTOR® Imagers.



Electrochemiluminescence Technology

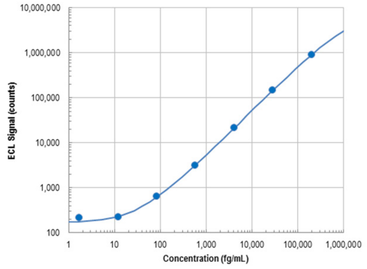
- Minimal non-specific background and strong responses to analyte yield high signal-to-background ratios.
- The stimulation mechanism (electricity) is decoupled from the response (light signal), minimizing matrix interference.
- Only labels bound near the electrode surface are excited, enabling non-washed 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 Analyte Information

Human Interleukin-17A (IL-17A) is a 155-amino acid, 35 kDa glycoprotein, biologically active as a disulfide-linked homodimer that adopts a cystine-knot fold. IL-17A is a pro-inflammatory cytokine secreted in response to the invasion of the immune system by extracellular pathogens and is associated with different disease conditions. It is secreted by the Th17 subset of T-helper cells and is induced by Interleukin 23 (IL-23). In addition to IL-17A, members of the IL-17 family include IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F. IL-17A is most similar to IL-17F with which it shares 50% sequence homology. Both IL-17A and IL-17F can exist as either biologically active homodimers (IL-17AA and IL-17FF) or biologically active heterodimers (IL-17AF). All three protein complexes are currently believed to signal through the same receptor complex, IL-17R. IL-17A has been linked to lung cancer, anti-tumor immunity, and allograft rejection, as well as multiple autoimmune diseases including rheumatoid arthritis, asthma, lupus, psoriasis, and multiple sclerosis.

We designed this study to measure the levels of IL-17A in serum samples from lung cancer patients and heavy smokers using MSD's ultrasensitive S-PLEX technology that can measure analytes in the fg/mL range.

4 Assay Performance

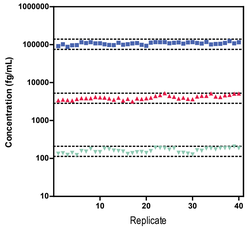


Conc. (fg/mL)	IL-17A ECL Signal (counts)
200,000	908,464
28,571	130,100
4,082	18,429
583	2,864
83	564
12	237
1.7	194
0	184

To determine limits of sensitivity and reproducibility of quality control (QC) samples for the assay kit lot, five replicates of each sample were measured over eight runs. The runs were performed by at least two operators over a minimum of four days (n=40). QC samples were prepared by spiking calibrator in diluent.

	IL-17A
Calibration Range	1.7 – 200,000 fg/mL
Hill Slope	1.00
Limit of Detection: Median (Range)	12 (7-15) fg/mL
Lot Specific Lower Limit of Quantitation	42 fg/mL
Lot Specific Upper Limit of Quantitation	160,000 fg/mL
Median concentration (95 serum or plasma samples from apparently healthy donors)	119 fg/mL
Percentage of normal serum/plasma within assay range (95 serum or plasma samples from apparently healthy donors)	93%

Limit of detection (LOD) is a calculated concentration corresponding to the average signal 2.5 standard deviations above the background (zero calibrator).
Lower limit of quantitation (LLOQ) and upper limit of quantitation (ULOQ) are established for the plate lot by measuring multiple levels of calibrator near the expected LLOQ and ULOQ. LLOQ and ULOQ are, respectively, the lowest and highest concentration of calibrator tested which has a %CV of 20% or less, with recovered concentration within 70-130%.



Three concentrations of calibrator diluted in the assay diluent were prepared as QC High, Mid, and Low. Dotted lines represent acceptable limits for QC values within the assay (± 30% of expected QC value).

The %CV for each source of variation was determined using analysis of variance (ANOVA) with guidance from NCCLS document EP05-A2.

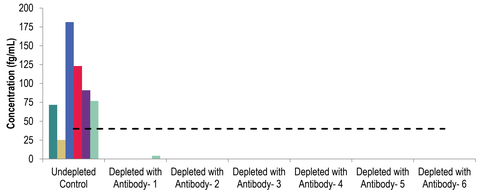
	Expected fg/mL	Average Measured fg/mL	% Recovery	%CV Within Run (n=40)	%CV Between Run (n=8)	%CV Between Day (n=4)	%CV Total
QC High	112,000	107,516	96	5.1	5.3	3.1	8.0
QC Mid	4,082	4,056	99	7.5	6.7	9.7	14.0
QC Low	167	161	97	8.2	10.6	5.9	14.7

These results suggest that the S-PLEX IL-17A Assay is highly sensitive, robust, and reproducible.

5 Specificity

Six unique IL-17A specific antibodies were selected to deplete IL-17A from six normal samples (three serum and three plasma samples). Depletion antibodies were conjugated to magnetic beads and then incubated with the samples for 2 hours at room temperature, followed by testing in the S-PLEX IL-17A assay.

Data are presented relative to the undepleted control samples. All the antibodies used resulted in IL-17A depletion by 95% or more in the six samples.



Analyte Concentration	IL-17A homodimer specific S-PLEX Assay Recombinant IL-17A	Recombinant IL-17A/F	Recombinant IL-17F
200 pg/mL	100%	<1%	<0.1%
20 pg/mL	100%	<1%	<0.1%

IL-17A and IL-17F homodimers, as well as IL-17A/F heterodimer, were measured in the S-PLEX IL-17A assay. Results show less than 1% cross-reactivity for both IL-17A/F and IL-17F calibrators at biologically relevant concentrations.

These results confirm that the S-PLEX IL-17A assay measures actual analyte levels and not just serum or plasma components that bind non-specifically to immunoassay components.

6 Dilution Linearity

Serum and EDTA plasma samples (n=6) were tested after 2, 4, or 8-fold dilutions. Average dilution linearity for the serum and EDTA plasma samples was 98% and 104%, respectively.

	Dilution Factor	Measured (fg/mL)	% Recovery
Serum 1	1x	7,080	-
	2x	3,340	94
	4x	1,433	81
	8x	597	67
Serum 2	1x	19,028	-
	2x	11,293	119
	4x	5,137	108
	8x	2,224	94
Serum 3	1x	3,146	-
	2x	1,485	94
	4x	858	109
	8x	459	117

Average %Recovery: 98

	Dilution Factor	Measured (fg/mL)	% Recovery
EDTA Plasma 1	1x	6,794	-
	2x	2,895	85
	4x	1,241	73
	8x	678	80
EDTA Plasma 2	1x	17,841	-
	2x	10,214	115
	4x	4,739	106
	8x	2,548	114
EDTA Plasma 3	1x	2,937	-
	2x	1,659	113
	4x	891	121
	8x	472	129

Average %Recovery: 104

Overall Average %Recovery: 101

%Recovery = Measured/(Expected)*100
Expected (fg/mL) = Undiluted Measured / Dilution Factor

These results suggest that there is minimal matrix effect in the S-PLEX IL-17A assay.

7 Spike Recovery

Serum and EDTA plasma samples (n=6) were tested unspiked, and spiked with calibrator at three concentrations. Average spike recoveries for the serum samples were 85% and for EDTA plasma samples were 88%.

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
Serum 1	unspiked	109	-
	10,000	8,696	86
	1,000	954	85
	100	185	76
Serum 2	unspiked	1,820	-
	10,000	13,296	115
	1,000	2,981	116
	100	1,946	126
Serum 3	unspiked	59	-
	10,000	6,284	62
	1,000	597	54
	100	106	47

Average %Recovery: 85

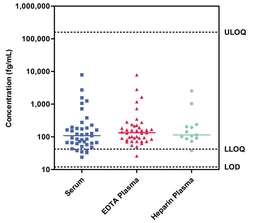
Overall Average %Recovery: 87

%Recovery = (Measured - Unspiked Measured) / Spike

	Spike (fg/mL)	Measured (fg/mL)	% Recovery
EDTA Plasma 1	unspiked	130	-
	10,000	7,665	75
	1,000	889	76
	100	232	102
EDTA Plasma 2	unspiked	1,780	-
	10,000	12,973	112
	1,000	2,685	91
	100	1,929	149
EDTA Plasma 3	unspiked	63	-
	10,000	6,842	68
	1,000	660	60
	100	122	59

Average % Recovery: 88

8 Levels in Normal Samples

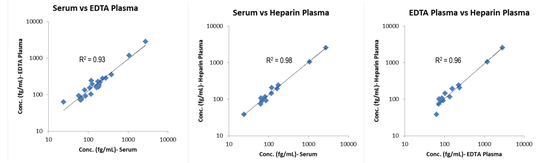


IL-17A levels were measured in 42 serum, 42 EDTA plasma, and 12 heparin plasma samples from apparently normal individuals.

These included 13 matched sets of serum, EDTA plasma, and heparin plasma samples, and 23 matched sets of serum and EDTA plasma samples.

The median IL-17A levels in all three sample types ranged between 100-120 fg/mL.

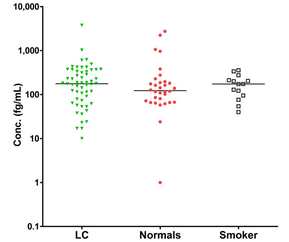
Note: Horizontal lines within each group indicate the median.



A good correlation ($R^2 > 0.93$) was observed between IL-17A levels in matched sets of serum, EDTA plasma, and heparin plasma samples.

9 Levels in Lung Cancer Serum

Serum samples from 59 lung cancer patients and 44 apparently healthy controls that included 14 heavy smokers were analyzed using S-PLEX technology to determine IL-17A concentrations. IL-17A serum concentrations were not found to be significantly different between lung cancer patients and controls, with a median concentration of 183 fg/mL and interquartile range (IQR) of 88-369 fg/mL (n=59) for lung cancer patients, compared to 128 fg/mL with an IQR of 75-198 fg/mL (n=44) for controls.



Note: Horizontal lines within each group indicate the median.

10 Conclusion

We have developed a highly specific and sensitive IL-17A assay using MSD's ultrasensitive S-PLEX technology that is 100 times more sensitive than the current limits of ELISA technology. This robust assay enables accurate determination of the IL-17A homodimer in serum and plasma samples from lung cancer patients and healthy controls. The results from this study do not support the use of IL-17A as an effective serum biomarker for the presence of lung cancer. Additionally, our data indicate that the median IL-17A level in normal sera is approximately 100 fg/mL, which is significantly lower than the previously reported value of greater than 5 pg/mL.

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