Method Validation of Quantitation IL-17A in Human Serum using the Ultrasensitive Quanterix Simoa™ Platform

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INTRODUCTION

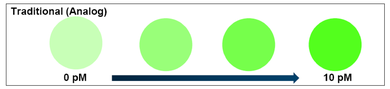
Interleukin17A (IL-17A) is disulfide-linked homo dimeric cytokine of 155 amino acids and a member of an IL-17 family of related cytokines. There is now compelling evidence that patients affected by autoimmune diseases have a higher incidence of several cardiovascular diseases. IL-17A plays a crucial role in the development of chronic inflammation and probably in the hemostatic disorders observed in patients with autoimmune diseases. The Simoa Human IL-17A 2.0 assay is a 3-step digital immunoassay for the quantitation of total IL-17A in human serum. The assay is designed to work with the Simoa HD-1 Analyzer which utilizes Single Molecule Array (SimoaTM) technology. The validation of method was conducted under theese assay parameters: including precision and accuracy, selectivity, linearity of dilution and stability. The assay sensitivity is 0.23438 pg/mL with MRD 1:4.

METHOD

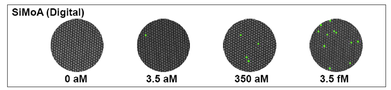
In the first step, anti-IL-17A coated paramagnetic capture beads were incubated with diluted samples standards and QC samples in order to capture IL-17A. The beads were washed and incubated with a biotinylated detection antibody that binds to the captured IL-17A. Following a second wash, a conjugate of streptavidin-ß-galactosidase (SBG) was added to the sample. SBG will bind to the biotinylated detection antibody, resulting in the enzymatic labeling of the captured IL-17A. Following a third wash, the capture beads were resuspended in a resorufin ß-D-galactopyranoside (RGP) substrate solution and transferred to the Simoa Disc. Individual capture beads are sealed within the microwells of the array. Captured and labeled IL-17A will hydrolyzes the RGP substrate into a fluorescent product that provides the signal for measurement.

A single labeled IL-17A molecule results in sufficient fluorescent signal in 30 seconds to be detected and counted by the Simoa optical system. At low IL-17A concentration, the percentage of bead-containing wells in the array that have a positive signal is proportional to the amount of IL-17A present in the sample. At higher IL-17A concentration, when most of the bead-containing wells have one or more labeled IL-17A molecules, the total fluorescence signal is proportional to the amount of IL-17A present in the sample. The concentration of IL-17A in unknown samples are interpolated from a standard curve.

The Advantage of the Simoa Assay

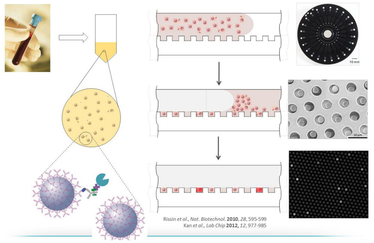


- Reaction volume = 100 ×10^{-6} L
- Diffusion = dilution = low sensitivity
- Millions of molecules needed to reach detection limit

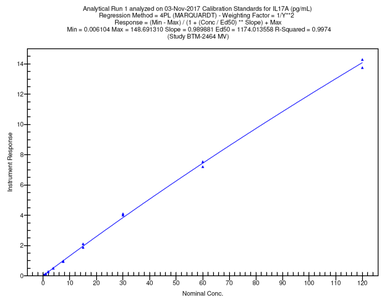


- Reaction volume = 50 ×10^{-15} L (2 billion times smaller)
- Diffusion defeated = single molecule resolution = ultimate sensitivity
- One molecule needed to reach detection limit

How The Simoa Assay Works



One Representative Standard Curve



Two Representative Standard Data

Run ID/Date		Std 11 0.11719 (pg/mL)	Std 10 0.23438 (pg/mL)	Std 9 0.46875 (pg/mL)	Std 8 0.93750 (pg/mL)	Std 7 1.87500 (pg/mL)	Std 6 3.75000 (pg/mL)	Std 5 7.50000 (pg/mL)	Std 4 15.00000 (pg/mL)	Std 3 30.00000 (pg/mL)	Std 2 60.00000 (pg/mL)	Std 1 120.00000 (pg/mL)
1/ 03Nov17	#1 (AEB)	0.10774	0.24243	0.46475	0.90288	1.89989	3.72128	7.32323	14.28726	32.05561	60.82252	116.88911
	#2 (AEB)	0.12407	0.26617	0.44224	0.92348	1.99671	3.67208	7.07461	16.11737	31.28421	57.96802	121.97742
	Intra run Mean	0.11591	0.25430	0.45350	0.91318	1.94830	3.69668	7.19892	15.20232	31.66991	59.39527	119.43327
	Intra run %CV	10.0	6.6	3.5	1.6	3.5	0.9	2.4	8.5	1.7	3.4	3.0
	Intra run %RE	-0.5	8.5	-3.3	-2.6	3.9	-1.4	-4.0	1.3	5.6	-1.0	-0.5
	n	2	2	2	2	2	2	2	2	2	2	2
2/ 06Nov17	#1 (AEB)	0.11063	0.25091	0.48192	0.86308	1.85600	3.43613	7.16569	15.98896	31.50756	65.18509	121.76664
	#2 (AEB)	0.11178	0.27481	0.47375	0.98269	1.86218	3.69922	7.21779	15.63052	28.98651	63.47983	109.83345
	Intra run Mean	0.11121	0.26286	0.47784	0.92289	1.85909	3.56768	7.19174	15.80974	30.24704	64.33246	115.80005
	Intra run %CV	0.7	6.4	1.2	9.2	0.2	5.2	0.5	1.6	5.9	1.9	7.3
	Intra run %RE	-2.6	12.2	1.9	-1.6	-0.8	-4.9	-4.1	5.4	0.8	7.2	-3.5
	n	2	2	2	2	2	2	2	2	2	2	2

IL-17A Selectivity in Human Serum

SeL Samples	Note	Serum lot	Spiked Il-17A Conc (pg/mL)	IL-17A Conc Found (pg/mL)	% Recovery	%CV
SeL01	Neat	BRH1164019	0.0000	0.04123	NA	**31.1**
SeL02	Neat	BRH1164020	0.0000	0.03124	NA	**31.6**
SeL03	Neat	BRH1164021	0.0000	0.02290	NA	**29.1**
SeL04	Neat	BRH1164022	0.0000	0.57251	NA	1.7
SeL05	Neat	BRH1164023	0.0000	0.07042	NA	15.3
SeL06	Neat	BRH1284973	0.0000	0.18349	NA	7.3
SeL07	Neat	BRH1284974	0.0000	0.03433	NA	8.5
SeL08	Neat	BRH1284975	0.0000	0.03523	NA	21.7
SeL09	Neat	BRH1284976	0.0000	0.09858	NA	9.4
SeL10	Neat	BRH1284977	0.0000	0.06242	NA	13.0
SeL11	LLOQ level	BRH1164019	0.23438	0.28148	102.5	4.8
SeL12	LLOQ level	BRH1164020	0.23438	0.30525	116.9	4.5
SeL13	LLOQ level	BRH1164021	0.23438	0.30727	121.3	2.5
SeL14	LLOQ level	BRH1164022	0.23438	0.77099	84.7	2.9
SeL15	LLOQ level	BRH1164023	0.23438	0.31275	103.4	1.0
SeL16	LLOQ level	BRH1284973	0.23438	0.47012	122.3	3.3
SeL17	LLOQ level	BRH1284974	0.23438	0.29389	110.7	10.3
SeL18	LLOQ level	BRH1284975	0.23438	0.28472	106.4	6.4
SeL19	LLOQ level	BRH1284976	0.23438	0.33241	99.8	6.4
SeL20	LLOQ level	BRH1284977	0.23438	0.25137	80.6	3.8

IL-17A in Matrix QC Stability

	Condition	LQC Conc. Found	LQC %CV	LQC % RE	HQC Conc. Found	HQC %CV	HQC % RE
Mean from six P&A Runs		0.44012	10.7	-1.9	101.78433	10.4	-1.9
Benchtop	QC1-AT	0.40768	5.2	-7.4	86.29682	0.6	-15.2
	QC2-AT	0.40507	3.4	-8.0	88.96826	7.9	-12.6
	QC3-AT	0.44179	7.9	0.4	105.44222	5.5	3.6
4°C Refrigerator	QC1-REF	0.42368	2.8	-3.7	106.91499	6.3	5.0
	QC2-REF	0.42996	8.0	-2.3	105.46283	6.0	3.6
	QC3-REF	0.46563	2.9	5.8	107.24412	0.7	5.4
Freeze Thaw 4X	QC1-FT4	0.44223	1.8	0.5	109.00536	5.3	7.1
	QC2-FT4	0.43702	3.1	-0.7	98.84938	3.5	-2.9
	QC3-FT4	0.40186	1.1	-8.7	101.48991	1.6	-0.3

CONCLUSIONS

Results and Conclusion: Method for quantitation of IL-17A in human serum samples has been validated using the Quanterix Simoa HD-1 Analyzer which has been validated following 21 CFR part 11. The LLOQ of the assay is 0.23438 pg/mL. The LQC evaluated in this validation was prepared at a concentration of 0.44012 pg/mL in human serum. The assay MRD is 1:4. The dynamic range of the method is 0.23438 - 120 pg/mL. Results for accuracy, precision, mixed samples, stability and dilution linearity met the required acceptance criteria specified in the validation plan.

REFERENCES

1. Rouvier E, Luciani MF, Mattéi MG, Denizot F, Golstein P (June 1993). "CTLA-8, cloned from an activated T cell, bearing AU-rich messenger RNA instability sequences, and homologous to a herpesvirus saimiri gene". Journal of Immunology. 150 (12): 5445–56.
2. IL-17A Data Sheet, Quanterix .