

SI Tables 1-5

Title of paper: Effects of Calibration Approaches on the Accuracy for LC-MS Targeted Quantification of Therapeutic Protein

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SI Table 1. Signature peptide (SP) candidates for HCV mAb^{a,b}

Peptide	Charge state	Precursor (m/z)	Top 5 product ions
Light Chain			
VDNALQSGNSQESVTEQDSK	2	1069.00	707.3, 1495.6, 893.4, 1408.8, 1022.5
TVAAPSVFIFPPSDEQLK	2	974.03	913.4, 1060.5, 802.6, 1320.7, 1173.6
VDNALQSGNSQESVTEQDSK	3	713.00	748.5, 641.3, 812.3, 606.3, 513.2
TVAAPSVFIFPPSDEQLK	3	649.29	457.4, 913.5, 517.4, 530.7, 443.8
VYACEVTHQGLSSPVTK	3	626.32	807.4, 772.2, 889.1, 692.0, 527.5
VYACEVTHQGLSSPVTK	2	938.97	788.4, 1154.7, 1053.5, 1088.5, 444.3
SSQSLVYSNGNTYLHWYLQKPGQSPK	3	995.17	1191.4,1241.0,1109.8,1066.3,1182.5
DSTYLSSTLTLSK	2	751.89	734.0, 836.5, 1036.5, 642.1, 448.2
DSTYLSSTLTLSK	3	501.60	489.7, 83.7, 477.4, 519.4, 448.2
SSQSLVYSNGNTYLHWYLQKPGQSPK	2	1492.31	613.3,1468.8,1331.7,1331.7,741.4
Heavy Chain			
GPSVFPLAPSSK	2	593.83	699.3,846.4,470.2,585.0,418.2
FNWYVDGVEVHNAK	3	559.94	708.8,616.0,534.3,262.1,766.6
FNWYVDGVEVHNAK	2	839.41	968.5,1067.5,697.3,1230.7,708.8
GGTGYWGQGTTLTVSSASTK	2	980.49	1337.7,780.3,1152.6,580.3,604.2
VVSVLTVLHQDWLNGK	3	603.68	805.4,712.7,656.2,531.6,762.2
GPSVFPLAPSSK	1	1186.65	699.4,751.4,769.5,846.4,418.2,
STSGGTAALGCLVK	2	661.35	576.3,760.4,689.3,567.5,519.3
VVSVLTVLHQDWLNGK	2	905.01	997.4,1110.5,617.3,1191.7,1310.7
TPEVTCVVVDVSHEDPEVK	3	714.02	834.6,472.3,856.9,906.7,970.9
FAFSLETSASTAYLQINNLIK	3	740.40	488.2,729.3,992.2,601.3,865.8,
GGTGYWGQGTTLTVSSASTK	3	653.66	681.5,580.3,493.2,641.0,804.1,780.4
TPEVTCVVVDVSHEDPEVK	2	1070.52	1667.7,472.3,1039.5,1154.5,940.5,1253.6
FAFSLETSASTAYLQINNLIK	2	1110.09	1005.5,842.4,729.4,601.4,1264.6
STSGGTAALGCLVK	3	441.23	576.3,588.4,373.6,633.1,567.5
TTPPVLDSDGSFFLYSK	2	937.98	836.7,1265.5,1378.8,827.4,1150.6
NEDTATYFCTR	2	689.30	583.2,847.4,746.3,1134.5,436.1
EEQYNSTYR	2	595.26	526.2,369.1,640.3,803.3,532.1
TTPPVLDSDGSFFLYSK	3	625.32	827.7,836.3,609.2,821.2,657.5
NEDTATYFCTR	1	1377.57	1019.4,1134.5,1001.6,1116.6,847.4

^a In total, more than 85% sequence coverage was achieved. Peptides that are not unique to HCV-mAb, or containing labile residues, known modification sites or missed cleavage were removed.

^b The top 2 most intensive charge states of each candidate were investigated. The peptide/charge combinations were sorted by the abundances of precursor peak, from high to low.

SI Table 2. Actual purities of the standards as quantified by quantitative amino acid analysis (AAA, N=6). All standards were labeled as >95% in purity by the providers.

	HCV-mAb (Full-length protein)	GPS	TVA	Extended-GPS	Extended-TVA
Mean	78%	90%	65%	75%	97%
RSD%	3%	9%	8%	6%	3%

SI Table 3. Validation of the peptide-, extended-peptide- and protein-level calibration approaches using QC spiked with *their respective calibrators* into blank plasma sample (N=6)^{a,b,c}.

Calibration approaches	Signature peptide	Range (µg/ml)	R ²	Accuracy			Precision		
				1.6 µg/ml	10 µg/ml	80 µg/ml	1.6 µg/ml	10 µg/ml	80 µg/ml
Protein calibrator with SIL-protein I.S. (Protein-spiked QC)	GPS	0.1~200	>0.9953	91%	96%	97%	3%	7%	6%
	TVA	0.1~200	>0.9932	90%	96%	106%	4%	9%	3%
Extended-peptide calibrator with SIL-extended-peptide I.S. (Extended-peptide-spiked QC)	GPS	0.1~200	>0.9963	98%	88%	98%	7%	3%	3%
	TVA	0.1~200	>0.9931	101%	86%	99%	5%	3%	3%
Peptide calibrator with SIL-peptide I.S. (Peptide-spiked QC)	GPS	0.1~200	>0.9974	96%	103%	103%	5%	6%	4%
	TVA	0.1~200	>0.9915	98%	96%	100%	7%	8%	5%

^a QC samples were prepared by spiking pure protein (before sample preparation, for protein calibration), synthesized extended-peptides (spiked before digestion, for extended-peptide calibration) or peptides (spiked after digestion, for peptide-level calibration) into blank rat plasma at three levels: 1.6, 10 and 80 µg/mL protein or **equal molarities** of peptides/extended peptides.

^b Each sample was analyzed three times in each of two different days (day 1 and day 14).

^c The purities of all standards were accurately measured by quantitative AAA procedure.

SI Table 4. Investigation of the accuracy and precision of the five calibration approaches using QC prepared by spiking pure protein into blank plasma (N=6)^{a, b, c}. The data is also illustrated in Fig. 3.

Calibration methods	Signature peptide	Range (µg/ml protein)	R ²	Accuracy			Precision		
				1.6 µg/ml	10 µg/ml	80 µg/ml	1.6 µg/ml	10 µg/ml	80 µg/ml
Protein calibrator with SIL-protein I.S.	GPS	0.1~200	>0.9953	91%	96%	97%	3%	7%	6%
	TVA	0.1~200	>0.9932	90%	96%	106%	4%	9%	3%
Protein calibrator with SIL-extended-peptide I.S.	GPS	0.1~200	>0.9945	93%	95%	92%	4%	6%	6%
	TVA	0.1~200	>0.9917	95%	94%	95%	7%	3%	3%
Protein calibrator with SIL-peptide I.S.	GPS	0.1~200	>0.9924	100%	99%	109%	6%	7%	0%
	TVA	0.1~200	>0.9912	94%	95%	103%	5%	8%	6%
Extended-peptide calibrator with SIL-extended-peptide I.S.	GPS	0.1~200	>0.9963	78%	74%	70%	4%	6%	6%
	TVA	0.1~200	>0.9931	58%	67%	65%	5%	3%	7%
Peptide calibrator with SIL-peptide I.S.	GPS	0.1~200	>0.9974	71%	68%	77%	4%	7%	2%
	TVA	0.1~200	>0.9915	38%	39%	41%	10%	8%	11%

^a QC samples were prepared by spiking pure protein into blank rat plasma at three levels: 1.6, 10 and 80 µg/mL.

^b Each sample was analyzed three times in each of two different days (day 1 and day 14).

^c The purities of all standards were accurately measured by a quantitative AAA procedure, to eliminate bias arising from inaccurate purity of standards.

SI Table 5. Quantitative data in the time course samples by five different calibration approaches (N=4 animals)

Time(h)	Protein calibrator with SIL-protein I.S.		Peptide calibrator with SIL-peptide I.S.		Extended-peptide calibrator with SIL-extended-peptide I.S.		Protein calibrator with SIL-peptide I.S.		Protein calibrator with SIL-extended-peptide I.S.	
	GPS	TVA	GPS	TVA	GPS	TVA	GPS	TVA	GPS	TVA
1	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)	Mean±SD ($\mu\text{g/ml}$)
2	48.32±1.31	49.98±7.69	33.72±8.78	20.2±0.65	37.99±2.85	30.41±1.82	44.19±11.50	51.36±1.66	49.46±3.73	45.33±2.73
4	43.87±2.77	43.97±2.43	31.72±2.27	18.15±2.04	36.58±1.82	29.49±1.56	41.57±2.97	46.08±5.22	47.62±2.39	43.95±2.33
6	39.92±2.93	40.98±5.03	31.27±1.45	15.90±1.22	32.23±3.59	26.88±2.83	40.98±1.90	40.31±3.14	41.91±4.70	40.04±4.24
12	37.34±5.08	34.5±2.19	26.24±5.23	15.01±1.46	27.54±4.50	21.51±4.95	35.96±3.46	38.03±1.19	35.77±5.90	32.00±7.41
24	31.14±2.87	29.01±3.56	22.42±4.41	13.39±1.32	23.05±3.26	17.48±2.50	29.38±2.77	33.89±0.96	29.88±5.58	25.97±3.73
48	24.31±2.20	23.67±3.49	17.18±3.36	9.64±0.85	16.42±1.59	12.90±1.57	23.48±2.73	24.27±2.17	21.18±2.08	19.78±1.27
72	17.41±1.89	19.29±2.32	14.97±1.00	7.16±0.87	14.38±0.88	10.78±0.94	19.62±0.91	17.96±1.53	18.51±0.75	16.58±1.01
120	15.45±1.56	16.56±2.73	13.62±1.78	6.15±0.59	12.52±1.02	8.37±1.29	17.05±0.95	15.37±1.52	16.07±1.12	14.18±0.67
168	13.26±0.92	12.99±1.27	10.12±0.58	5.64±0.54	10.41±0.94	7.07±1.08	13.26±0.76	14.06±1.12	13.3±0.93	11.71±0.63
216	11.13±0.62	11.10±0.71	9.83±0.41	4.45±0.53	8.51±0.59	5.79±0.90	12.88±0.21	11.01±0.59	10.82±0.77	10.10±0.80
264	9.77±0.64	10.89±0.48	7.89±0.37	4.06±0.54	7.88±0.48	5.23±0.79	10.73±0.55	10.01±1.38	9.99±0.63	9.06±0.69
360	9.26±0.86	8.70±0.74	7.36±0.43	3.53±0.48	7.49±0.50	5.16±0.56	9.65±0.31	8.65±1.23	9.49±0.33	7.99±0.48
504	6.41±0.44	5.62±0.61	5.04±0.29	2.48±0.27	5.17±0.47	3.23±0.55	6.61±0.38	5.91±0.43	6.56±0.75	6.68±0.67
648	4.91±0.32	4.49±0.40	3.34±0.52	1.76±0.29	3.41±0.36	2.12±0.50	4.38±0.48	4.05±0.42	4.81±0.57	4.52±0.67
	3.19±0.34	3.07±0.35	2.43±0.49	1.41±0.23	2.70±0.36	1.40±0.32	3.19±0.47	3.16±0.34	3.39±0.35	3.12±0.43