

Supplemental Information

Compound	Retention Time (min)	RT Window (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	Min Dwell Time (ms)
DEALNNR(+2)	2	1	416.201	516.289	14.97	43	48.449
DEALNNR(+2)	2	1	416.201	587.326	14.59	43	48.449
DEALNNR (medium)(+2)	2	1	419.211	522.309	14.97	43	48.449
DEALNNR (medium)(+2)	2	1	419.211	593.346	14.59	43	48.449
DEALNNR (heavy)(+2)	2	1	421.206	526.297	14.97	43	48.449
DEALNNR (heavy)(+2)	2	1	421.206	597.334	14.59	43	48.449
ASANLAATK(+2)	2.94	1	423.737	617.362	14.1	60	10.409
ASANLAATK(+2)	2.94	1	423.737	688.399	12.6	60	10.409
ASANLAATK (medium)(+2)	2.94	1	425.75	621.387	14.1	60	10.409
ASANLAATK (medium)(+2)	2.94	1	425.75	692.424	12.6	60	10.409
ASANLAATK (heavy)(+2)	2.94	1	427.744	625.376	14.1	60	10.409
ASANLAATK (heavy)(+2)	2.94	1	427.744	696.413	12.6	60	10.409
STQAAIDQINGK	3.1	1	623.325	787.43	18.42	85	10.409
STQAAIDQINGK	3.1	1	623.325	858.467	19	85	10.409
STQAAIDQINGK (medium)(+2)	3.1	1	625.328	791.438	18.42	85	10.409
STQAAIDQINGK (medium)(+2)	3.1	1	625.328	862.475	19	85	10.409
STQAAIDQINGK (heavy)(+2)	3.1	1	627.334	795.445	18.42	85	10.409
STQAAIDQINGK (heavy)(+2)	3.1	1	627.334	866.482	19	85	10.409
GYSFTTTAER (medium)(+2)	3.18	1	569.777	684.362	17.85	68	10.409
GYSFTTTAER (medium)(+2)	3.18	1	569.777	918.462	17.05	68	10.409
GYSFTTTAER (heavy)(+2)	3.18	1	571.771	688.35	17.85	68	10.409
GYSFTTTAER (heavy)(+2)	3.18	1	571.771	922.45	17.05	68	10.409
GYSFTTTAER (superheavy)(+2)	3.18	1	573.774	692.357	17.85	68	10.409
GYSFTTTAER (superheavy)(+2)	3.18	1	573.774	926.458	17.05	68	10.409
VTLADAGFIK(+2)	5.1	1	517.797	721.388	15.4	50	48.426
VTLADAGFIK(+2)	5.1	1	517.797	834.472	13.9	50	48.426
VTLADAGFIK (medium)(+2)	5.1	1	519.81	725.413	15.4	50	48.426
VTLADAGFIK (medium)(+2)	5.1	1	519.81	838.497	13.9	50	48.426
VTLADAGFIK (heavy)(+2)	5.1	1	521.804	729.402	15.4	50	48.426
VTLADAGFIK (heavy)(+2)	5.1	1	521.804	842.486	13.9	50	48.426
FLPFQQFGR(+2)	6.38	1	570.303	440.227	15.5	70	98.331
FLPFQQFGR(+2)	6.38	1	570.303	879.447	17	70	98.331
FLPFQQFGR (medium)(+2)	6.38	1	573.313	443.237	15.5	70	98.331
FLPFQQFGR (medium)(+2)	6.38	1	573.313	885.467	17	70	98.331
FLPFQQFGR (heavy)(+2)	6.38	1	575.307	445.231	15.5	70	98.331
FLPFQQFGR (heavy)(+2)	6.38	1	575.307	889.455	17	70	98.331

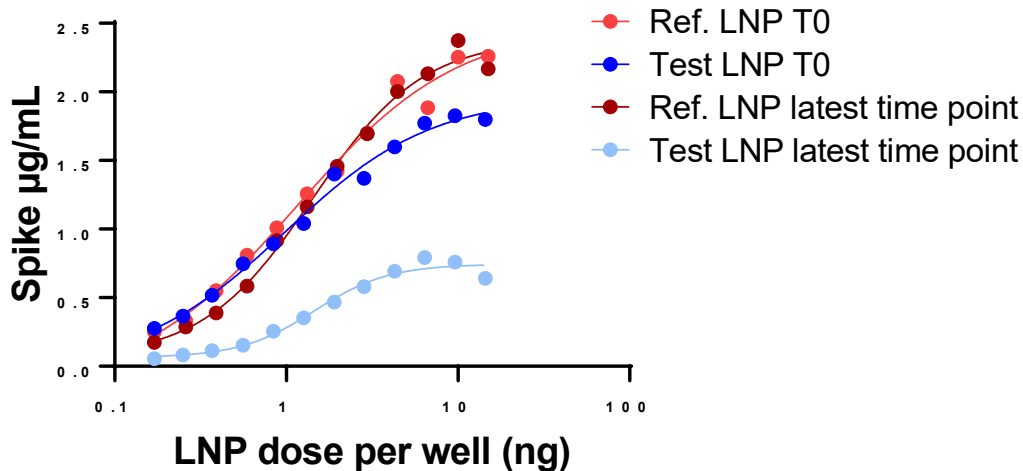
Supplemental Table 1: MRM peptide parameters for covid Spike, influenza H3 (the IDC), and beta actin proteins.

Compound	Retention Time (min)	RT Window (min)	Precursor (m/z)	Product (m/z)	Collision Energy (V)	RF Lens (V)	Min Dwell Time (ms)
STQAAIDQISGK(+2)	4.36	2	609.819	831.457	18.2	85	31.706
STQAAIDQISGK(+2)	4.36	2	609.819	902.494	19.7	85	31.706
STQAAIDQISGK (medium)(+2)	4.36	2	611.832	835.482	18.2	85	31.706
STQAAIDQISGK (medium)(+2)	4.36	2	611.832	906.519	19.7	85	31.706
STQAAIDQISGK (heavy)(+2)	4.36	2	613.826	839.471	18.2	85	31.706
STQAAIDQISGK (heavy)(+2)	4.36	2	613.826	910.508	19.7	85	31.706
LNVEDTAEIR(+2)	4.7	2	630.825	228.134	23	80	31.706
LNVEDTAEIR(+2)	4.7	2	630.825	934.448	22	80	31.706

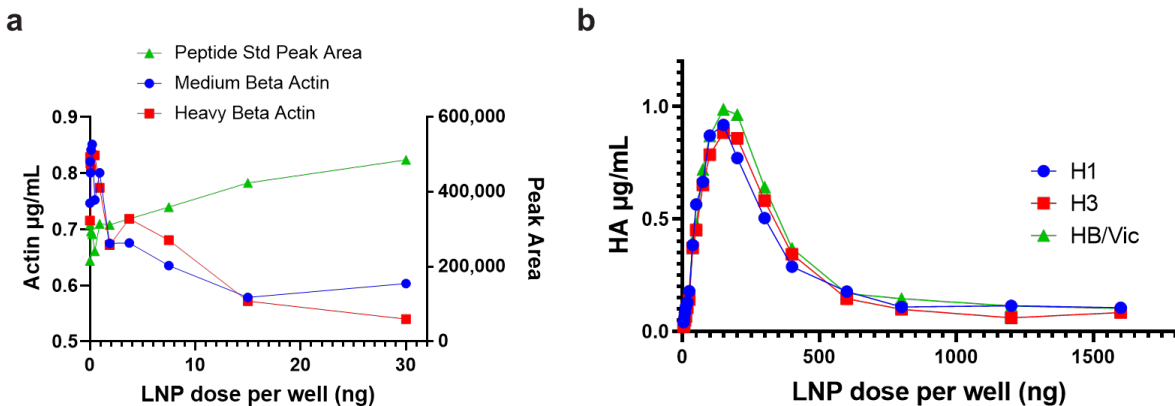
LNVEDTAEIR (medium)(+2)	4.7	2	633.835	228.134	23	80	31.706
LNVEDTAEIR (medium)(+2)	4.7	2	633.835	940.468	22	80	31.706
LNVEDTAEIR (heavy)(+2)	4.7	2	635.829	228.134	23	80	31.706
LNVEDTAEIR (heavy)(+2)	4.7	2	635.829	944.456	22	80	31.706
EQLSSVSSFER(+2)	4.8	2	634.809	625.294	18.04	82	31.706
EQLSSVSSFER(+2)	4.8	2	634.809	898.426	21.83	82	31.706
EQLSSVSSFER (medium)(+2)	4.8	2	637.819	631.314	18.04	82	31.706
EQLSSVSSFER (medium)(+2)	4.8	2	637.819	904.447	21.83	82	31.706
EQLSSVSSFER (heavy)(+2)	4.8	2	639.823	635.321	18.04	82	31.706
EQLSSVSSFER (heavy)(+2)	4.8	2	639.823	908.454	21.83	82	31.706
LVLATGLR(+2)	7	2	421.776	517.309	14.77	56	31.743
LVLATGLR(+2)	7	2	421.776	630.393	13.55	56	31.743
LVLATGLR (medium)(+2)	7	2	424.786	523.329	14.77	56	31.743
LVLATGLR (medium)(+2)	7	2	424.786	636.413	13.55	56	31.743
LVLATGLR (heavy)(+2)	7	2	426.78	527.318	14.77	56	31.743
LVLATGLR (heavy)(+2)	7	2	426.78	640.402	13.55	56	31.743
GDVFVIR(+2)	7.1	2	403.231	387.271	13	49	31.709
GDVFVIR(+2)	7.1	2	403.231	534.34	13	49	31.709
GDVFVIR (medium)(+2)	7.1	2	406.242	393.292	13	49	31.709
GDVFVIR (medium)(+2)	7.1	2	406.242	540.36	13	49	31.709
GDVFVIR (heavy)(+2)	7.1	2	408.236	397.28	13	49	31.709
GDVFVIR (heavy)(+2)	7.1	2	408.236	544.348	13	49	31.709
NLNSLSELEVK	8.3	2	623.338	904.498	18	99	31.709
NLNSLSELEVK	8.3	2	623.338	1018.541	19.21	99	31.709
NLNSLSELEVK (medium)(+2)	8.3	2	625.341	908.506	18	99	31.709
NLNSLSELEVK (medium)(+2)	8.3	2	625.341	1022.549	19.21	99	31.709
NLNSLSELEVK (heavy)(+2)	8.3	2	627.345	912.513	18	99	31.709
NLNSLSELEVK (heavy)(+2)	8.3	2	627.345	1026.556	19.21	99	31.709
ILFIEEGK(+2)	9	2	474.773	199.18	17	61	31.709
ILFIEEGK(+2)	9	2	474.773	722.372	14	61	31.709
ILFIEEGK (medium)(+2)	9	2	476.777	199.18	17	61	31.709
ILFIEEGK (medium)(+2)	9	2	476.777	726.379	14	61	31.709
ILFIEEGK (heavy)(+2)	9	2	478.78	199.18	17	61	31.709
ILFIEEGK (heavy)(+2)	9	2	478.78	730.386	14	61	31.709

Supplemental Table 2: MRM peptide parameters for influenza H1, H3, HB/Victoria, N1, N2, NB/Victoria, and H2 (the IDC) proteins.

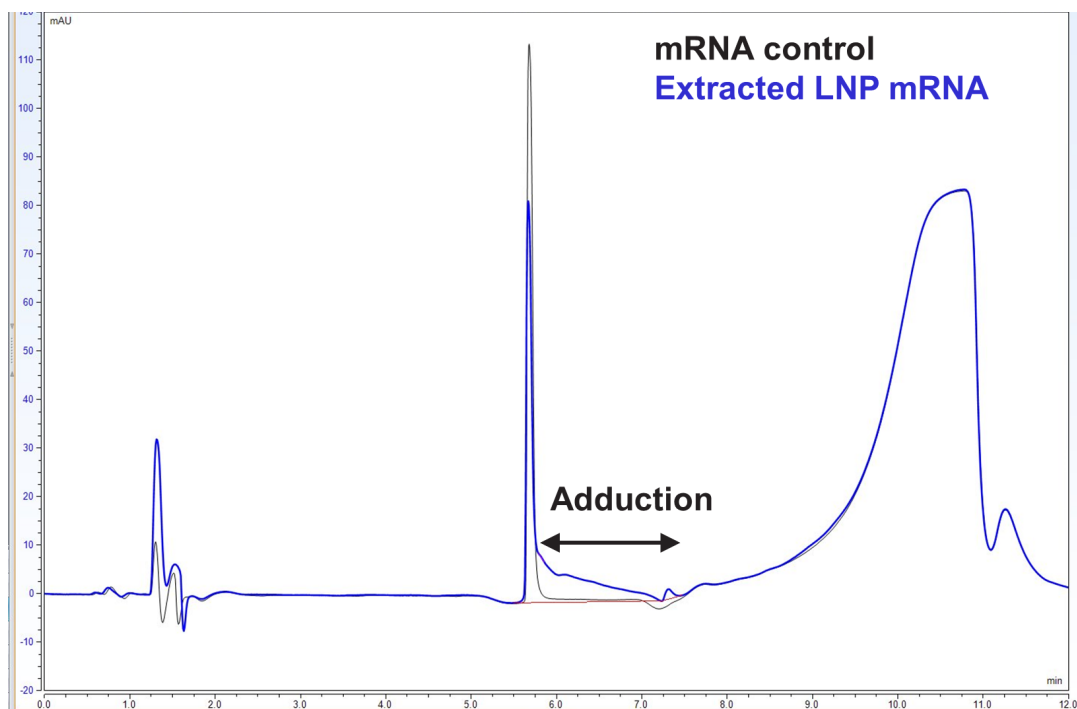
of any HA subtypes. Transfected Flu LNPs resulted in the quantification of only H1 (purple), H3 (blue), HB/Victoria (green), and HB/Victoria (pink) antigens, with no S signal. A Flu and Covid LNP mixture resulted in quantitation of S, H1, H3, HB/Victoria, and HB/Yamagata antigens.



Supplemental Figure 3: Loss of parallelism in the aged LNP material. A comparison of the pSIM dose response between time zero (T0, dark blue) and the latest stability time point of aged Covid test LNP (light blue) materials shows a loss of parallelism with a decrease in Emax. Non-aged reference LNP material for both sample sets (dark and light red) displays similar dose responses, indicating the variable dose response for the test stability sample is not the result of run-to-run variability.



Supplemental Figure 4: Beta actin housekeeping protein decrease and the antigen “hook effect” with increasing LNP dose per well. The amount of expressed beta actin from Hep3b cells transfected with Covid LNP is quantified using a spiked “superheavy” beta actin peptide internal standard (a). A clear decrease in medium and heavy beta actin concentration is observed as the LNP dose per well increases. The superheavy peptide standard does not show similar decrease in peak area, confirming that the drop in medium and heavy actin is from the sample itself, rather than instrument variability. BHK21 cells transfected with TIV LNP result in a decrease in H1, H3, and HB/Vic expression as the LNP dose increases, termed the “hook effect” (b).



Supplemental Figure 5: LC-UV trace of lipid adduction. Lipid adduction of a poor quality mRNA extracted from LNP drug product (blue) is visible in the LC-UV 260nm trace following a main mRNA peak, as confirmed with a mRNA-only control (black).

Parameter	Target Criteria	Result
Precision (Repeatability)	RSD $\leq$ 25%	LNP 1:1:1 %RSD
		H1 = 12.7%
		H3 = 9.0%
		HB = 12.2%
		LNP 1:1:4 %RSD
		H1 = 12.9%
Precision (Intermediate Precision)	RSD $\leq$ 30%	LNP 1:1:1 %RSD
		H1 = 11.7%
		H3 = 9.3%
		HB = 13.8%
		LNP 1:1:4 %RSD
		H1 = 11.9%
Accuracy	70% - 130%	LNP 1:1:1 Mean RP%
		H1 = 99%

		H3 = 88%
		HB = 93%
		LNP 1:1:1 R ² Mean
Linearity	R ² ≥ 0.90	H1 M = 0.9748
		H3 M = 0.9729
		HB M = 0.9693
		H1 H = 0.9672
		H3 H = 0.9761
		HB H = 0.9602
		LNP 1:1:4 R ² Mean
		H1 M = 0.9457
		H3 M = 0.9535
		HB M = 0.9328
		H1 H = 0.9694
		H3 H = 0.9792
		HB H = 0.9529

Supplemental Table 3: pSIM method performance per ICH guidelines using BHK21 cells transfected with Influenza sa-mRNA LNPs. Two seasonal influenza LNPs containing different subtype ratios (1:1:1 or 1:1:4) were assessed for precision, accuracy, linearity, and specificity. Repeatability and intermediate precision of six replicates of both 1:1:1 and 1:1:4 formulations yielded a %RSD for each H1, H3, and HB subtype. Accuracy was evaluated by determining the relative potency of the 1:1:1 LNP against itself, with an expected relative potency of 100%. Linearity was measured for the H1, H3, and HB subtypes across six replicates of the two LNP formulations. A specificity assessment identified no MRM peptide signal for alternative LNP HA peptides or blank cryobuffer.