

Supplementary Material

(RP)UHPLC/UV analytical method to quantify dsRNA during mRNA vaccine manufacturing process

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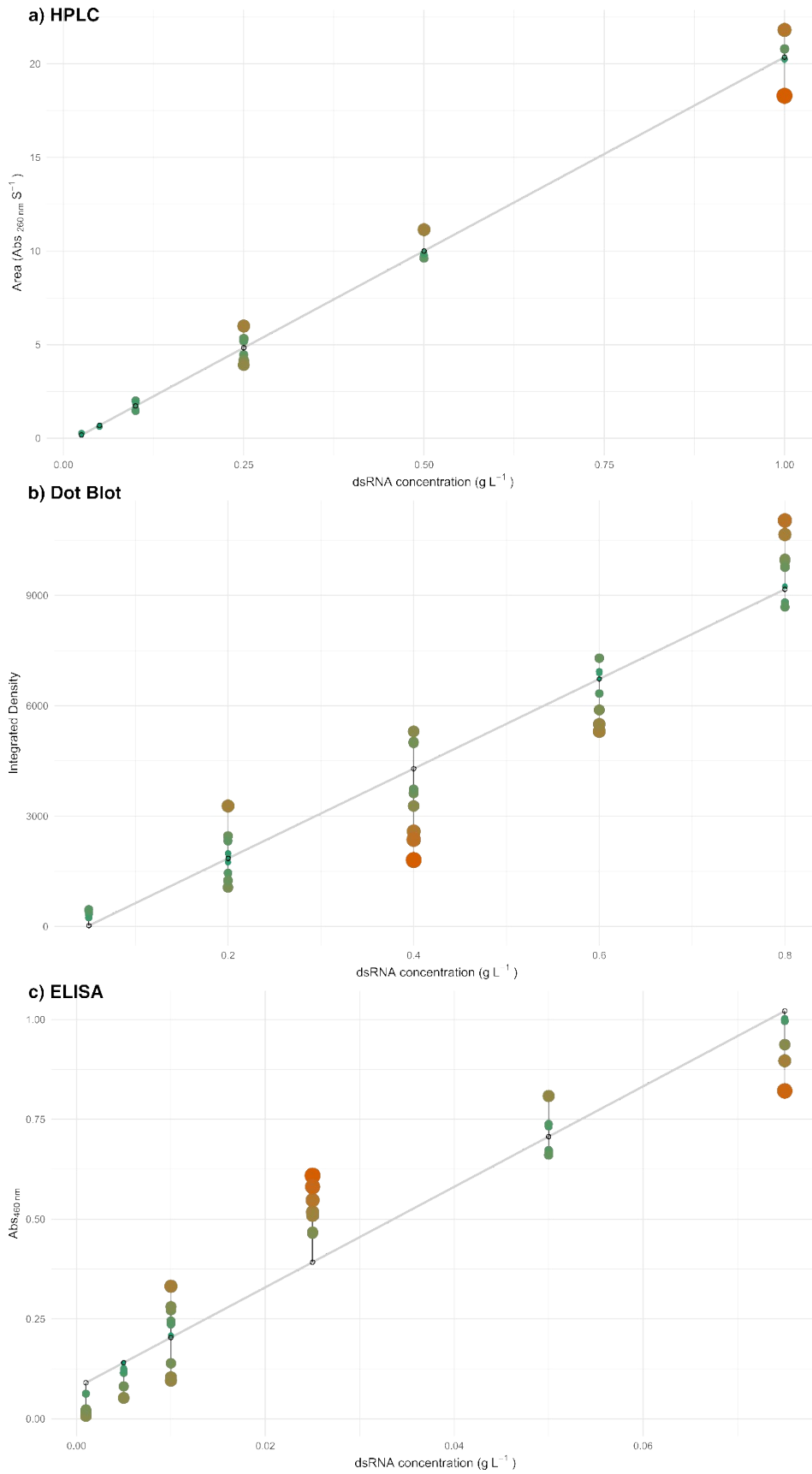
These authors contributed equally to this work

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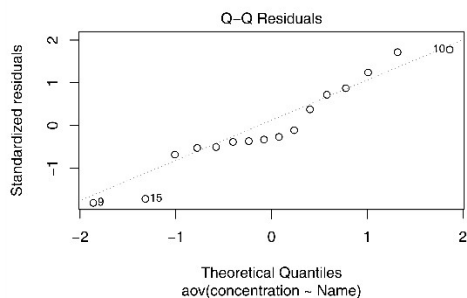
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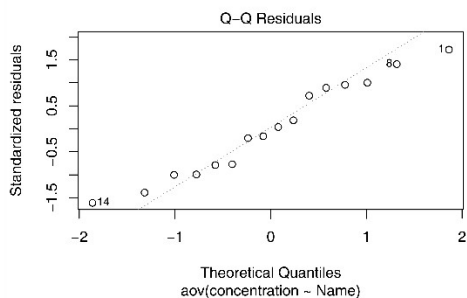


Supplementary Figure S1. Residual plots for the calibration curves used to calculate dsRNA concentration using the HPLC (a), the Dot Blot (b) and ELISA (c).

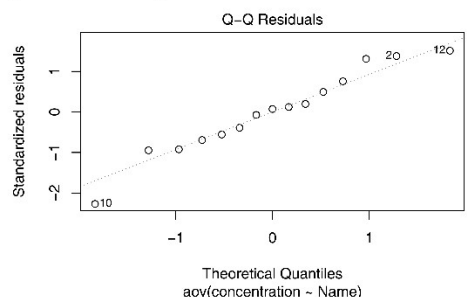
a) HPLC low_RNA



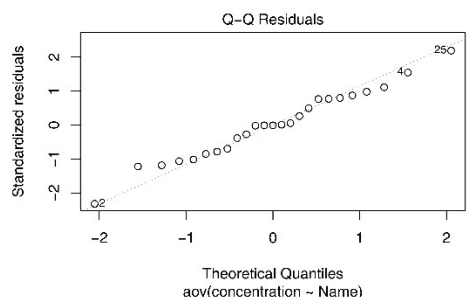
b) HPLC high_RNA



c) Dot Blot low_RNA



d) Dot Blot high_RNA



Supplementary Figure S2. Residual plots for the dsRNA spiking studies. Dot Blot results using 0.1 g L⁻¹ (a) and 0.4 g L⁻¹ dsRNA (b). HPLC results using 0.1 g L⁻¹ (c) and 0.4 g L⁻¹ of dsRNA (d).

Supplementary Table S1. Dot Blot Tukey test results for high dsRNA concentration (0.4 g.L⁻¹). Concentration used for DNA correspond to 10 and 90 nM and to NTPs to 2 and 7.6 mM, for low and high, respectively.

Factor 1	Factor 2	Mean difference	Confidence Interval at 95%		Adjusted P value
			Lower bound	Upper bound	
DNA_high	DNA_low	0.169	-0.063	0.400	0.228
	NTP_high	0.319	0.108	0.531	0.002
	NTP_low	0.042	-0.170	0.253	0.975
	RNA_high	0.008	-0.212	0.228	1.000
DNA_low	NTP_low	0.151	-0.061	0.362	0.246
	DNA_low	-0.127	-0.338	0.085	0.404
	RNA_high	-0.161	-0.381	0.059	0.223
NTP_low	NTP_high	-0.277	-0.467	-0.088	0.002
RNA_high	NTP_high	-0.312	-0.510	-0.113	0.001
	NTP_low	-0.034	-0.233	0.164	0.985

Supplementary Table S2. Dot BlotTukey test results for low dsRNA concentration. (0.1 g.L⁻¹). Concentration used for DNA correspond to 10 and 90 nM and to NTPs to 2 and 7.6 mM, for low and high, respectively.

Factor 1	Factor 2	Confidence Interval at 95%			Adjusted P value
		Mean Difference	Lower bound	Upper bound	
DNA_high	DNA_low	-0.004	-0.052	0.044	0.998
	NTP_high	-0.013	-0.058	0.032	0.864
	NTP_low	-0.022	-0.076	0.031	0.663
	RNA_low	-0.034	-0.082	0.014	0.213
DNA_low	NTP_low	-0.009	-0.054	0.036	0.965
	DNA_low	-0.018	-0.071	0.036	0.808
	RNA_low	-0.03	-0.078	0.018	0.322
NTP_low	NTP_high	-0.009	-0.060	0.042	0.974
RNA_low	NTP_high	-0.021	-0.066	0.024	0.571
	NTP_low	-0.012	-0.065	0.042	0.947

Supplementary Table S3. HPLC Tukey test results for high dsRNA concentration (0.4 g.L⁻¹). Concentration used for DNA correspond to 10 and 90 nM and to NTPs to 2 and 7.6 mM, for low and high, respectively.

Factor 1	Factor 2	Confidence Interval at 95%			Adjusted P value
		Mean Difference	Lower bound	Upper bound	
DNA_high	DNA_low	-0.014	-0.139	0.111	0.996
	NTP_high	-0.041	-0.157	0.075	0.775
	NTP_low	0.02	-0.105	0.145	0.984
	RNA_high	0.014	-0.129	0.156	0.998
DNA_low	NTP_low	-0.028	-0.153	0.098	0.950
	DNA_low	0.034	-0.100	0.168	0.919
	RNA_high	0.027	-0.122	0.177	0.974
NTP_low	NTP_high	0.061	-0.064	0.187	0.534
RNA_high	NTP_high	0.055	-0.087	0.197	0.724
	NTP_low	-0.007	-0.156	0.143	1.000

Supplementary Table S4. HPLC Tukey test results for low dsRNA concentration (0.1 g.L⁻¹). Concentration used for DNA correspond to 10 and 90 nM and to NTPs to 2 and 7.6 mM, for low and high, respectively.

Factor 1	Factor 2	Confidence Interval at 95%			Adjusted P value
		Mean Difference	Lower bound	Upper bound	
DNA_high	DNA_low	0.011	-0.011	0.033	0.503
	NTP_high	-0.003	-0.027	0.021	0.992
	NTP_low	0.007	-0.017	0.031	0.874
	RNA_low	0.009	-0.018	0.036	0.824
DNA_low	NTP_low	-0.014	-0.038	0.010	0.352
	DNA_low	-0.004	-0.028	0.020	0.976
	RNA_low	-0.002	-0.029	0.025	0.998
NTP_low	NTP_high	0.010	-0.015	0.036	0.710
RNA_low	NTP_high	0.012	-0.017	0.040	0.666
	NTP_low	0.002	-0.027	0.030	0.999