DNA contamination in 8 vials of Pfizer monovalent mRNA vaccines

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Introduction

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DNA contamination has recently been documented in mono and bivalent mRNA vaccines. This study was limited in the number of vials surveyed. We surveyed 8 vials from the same lot of Pfizer monovalent mRNA vaccines (lot # FL8095) using qPCR and RT-qPCR. These vials were unopened but dated 9/21 on the label and 3/4/22 (hand writing). 2ul from each vial were analyzed.

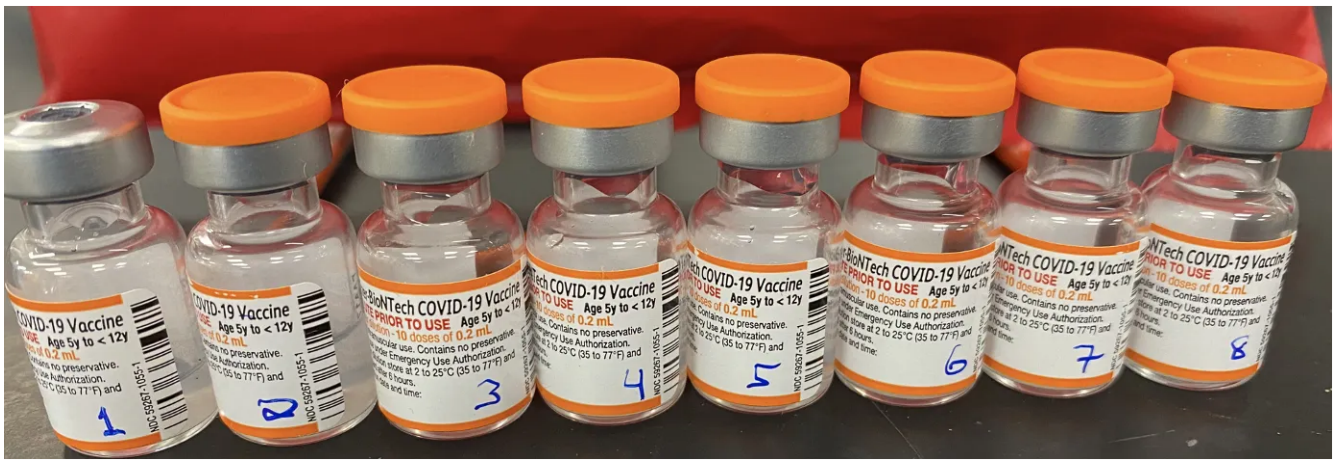


Figure 1. 8 Pfizer monovalent vaccines used in this study

Results

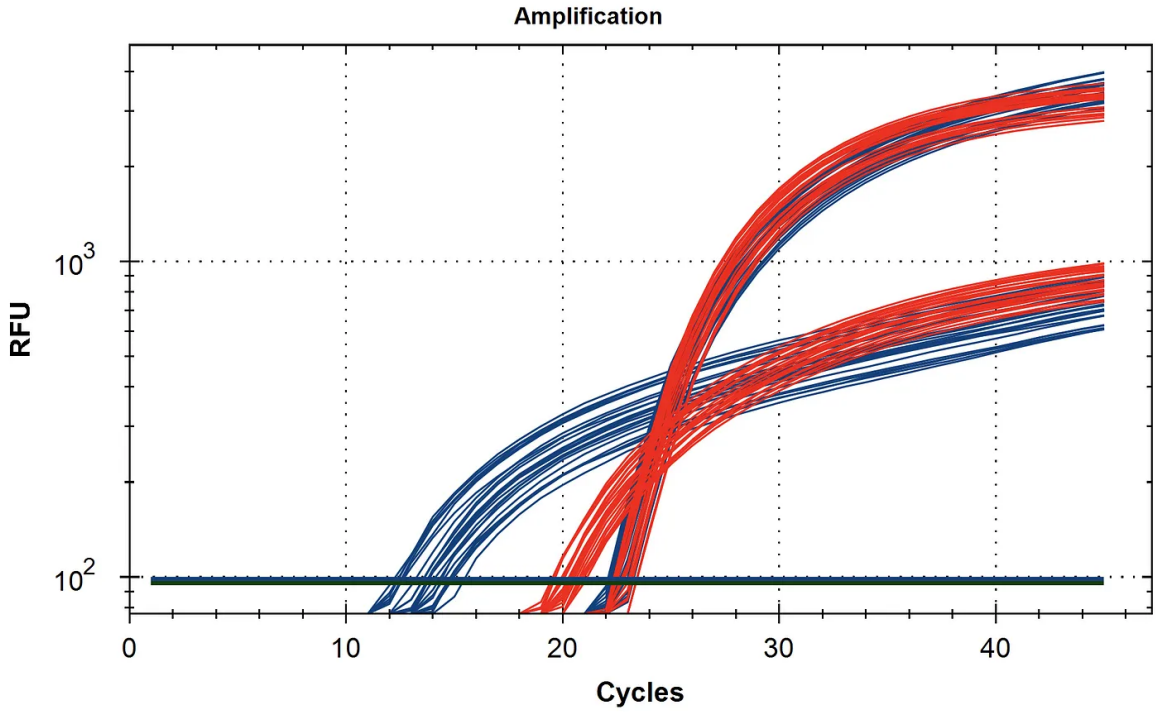


Figure 2. qPCR and RT-qPCR of 8 vials run in triplicate. Red is Vector. Blue is Spike

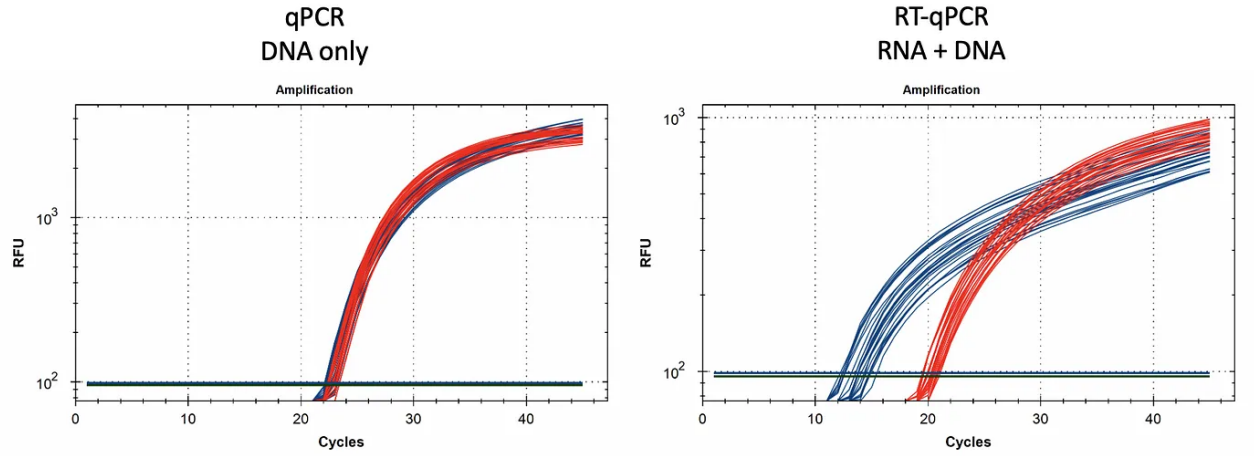


Figure 3. qPCR and RT-qPCR split out into qPCR (left) and RT-qPCR (right). Red is vector and blue is spike.

qPCR-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.12	22.98	22.58	22.33	22.36	22.08	22.20	22.06	0.401
Replicate 2	23.16	22.90	22.70	22.36	22.20	22.16	22.29	22.22	0.373
Replicate 3	23.22	22.84	22.59	22.29	22.44	22.26	22.29	22.11	0.366
STDEV	0.05	0.07	0.07	0.03	0.12	0.09	0.05	0.08	

qPCR: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	0.20	0.08	0.27	(0.00)	0.18	0.18	0.10	0.24	0.090
Replicate 2	0.16	0.22	0.29	0.11	0.18	0.12	0.03	0.13	0.079
Replicate 3	0.14	0.31	0.20	0.17	0.31	0.19	0.20	0.13	0.069
STDEV	0.03	0.11	0.05	0.09	0.08	0.04	0.08	0.06	

qPCR-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	23.33	23.06	22.85	22.32	22.54	22.26	22.30	22.30	0.411
Replicate 2	23.32	23.12	23.00	22.47	22.38	22.28	22.32	22.35	0.419
Replicate 3	23.36	23.15	22.79	22.46	22.75	22.46	22.49	22.23	0.383
STDEV	0.02	0.04	0.11	0.08	0.19	0.11	0.11	0.06	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	1	1	1	1	1	1	1	1	0.068
Replicate 2	1	1	1	1	1	1	1	1	0.062
Replicate 3	1	1	1	1	1	1	1	1	0.056
STDEV	0.0	0.1	0.0	0.1	0.1	0.0	0.1	0.1	

Table 1. CT values for Spike and Vector during qPCR (DNA only). Standard deviation for triplicate measurements run horizontally in black font. Standard deviation for vial to vial run vertically in Red. Delta CT or (Vector CT minus Spike CT) represents the ratio of Spike to Vector DNA. Should = 1.

RT-Spike	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	14.05	14.77	13.18	13.77	13.79	12.52	12.62	13.53	0.749
Replicate 2	14.29	14.74	14.38	14.82	13.78	13.82	12.57	12.38	0.925
Replicate 3	14.49	14.91	15.43	13.84	13.74	13.55	12.36	12.19	1.141
STDEV	0.22	0.09	1.12	0.59	0.02	0.69	0.14	0.72	

RT: (Vector-Spike)	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	6.74	5.93	7.20	6.40	6.51	7.31	7.33	5.97	0.570
Replicate 2	6.33	6.06	5.92	5.67	6.34	6.13	6.92	7.06	0.478
Replicate 3	6.33	6.07	5.43	6.39	6.13	6.38	7.09	7.18	0.562
STDEV	0.24	0.07	0.91	0.42	0.19	0.62	0.21	0.67	

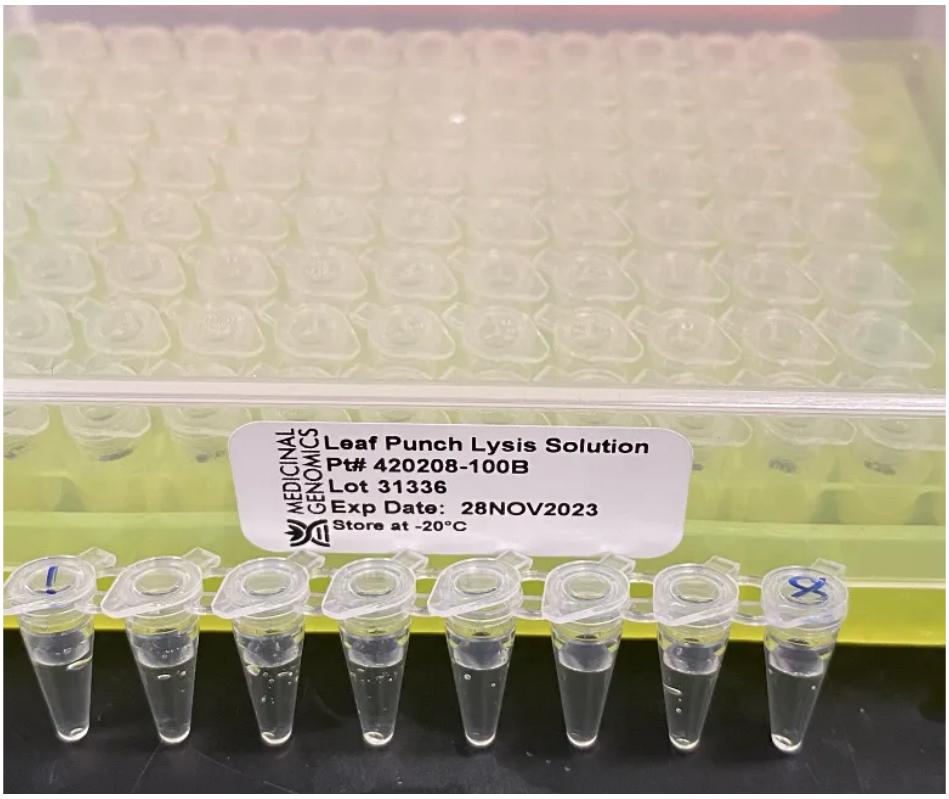
RT-Vector	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	20.80	20.71	20.39	20.16	20.30	19.83	19.95	19.50	0.439
Replicate 2	20.62	20.80	20.30	20.49	20.12	19.96	19.49	19.45	0.499
Replicate 3	20.81	20.98	20.86	20.23	19.88	19.93	19.45	19.37	0.638
STDEV	0.11	0.14	0.30	0.17	0.21	0.07	0.28	0.07	

RATIO RNA/DNA	Vial 1	Vial 2	Vial 3	Vial 4	Vial 5	Vial 6	Vial 7	Vial 8	STDEV
Replicate 1	107	61	147	84	91	159	161	63	41.54
Replicate 2	80	67	61	51	81	70	121	134	29.25
Replicate 3	80	67	43	84	70	83	136	145	34.79
STDEV	15.5	3.3	55.8	19.2	10.4	47.9	20.3	44.6	

Table 2. CT values for Spike and Vector during RT-qPCR (RNA+DNA). Ratio of RNA:DNA ranges from 43:1-161:1. EMA allowable range is 3030:1. 18-70 Fold over the EMA limit.

Methods

2ul from each vial was added to 100ul of Leaf Lysis Solution and boiled as previously described. 1ul of each boiled sample was used in qPCR and RT-qPCR as described previously.



2ul of Vaccine was added to 100ul of Leaf Punch Lysis Solution.

- Rapid Boil Prep for vaccine surveillance.
- qPCR methods previously described.

Conclusions

8/8 monovalent vaccines sourced from a single case from a single lot of Pfizer monovalent vaccines all fail the EMA specification of 3030:1 RNA:DNA (330ng/mg DNA/RNA). They are over the limit by an order of magnitude (18-70 fold). The DNA contamination is very consistent and the vial to vial ratio of RNA:DNA is very consistent within the same lot of monovalent vaccines. Further sequencing is underway to evaluate the 72bp heteroplasmic indel in the SV40 promoter of the vector. This was detected in the Pfizer bivalent vaccines.

References

FDA presentation on dsDNA contamination

Issues associated with residual cell-substrate DNA in viral vaccines

Issues associated with residual cell-substrate DNA in viral vaccines

Li Sheng-Fowler, Andrew M. Lewis Jr., Keith Peden

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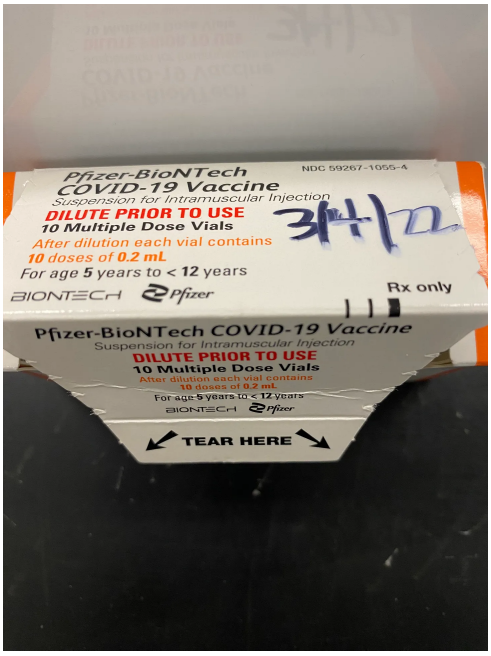
Abstract

The presence of some residual cellular DNA derived from the production-cell substrate in viral vaccines is inevitable. Whether this DNA represents a safety concern, particularly if the cell substrate is derived from a tumor or is tumorigenic, is unknown. DNA has two biological activities that need to be considered. First, DNA can be oncogenic; second, DNA can be infectious. As part of our studies to assess the risk of residual cell-substrate DNA in viral vaccines, we have established assays that can quantify the biological activities of DNA. From data obtained using these assays, we have estimated the risk of an oncogenic or an infectious event from DNA. Because these estimates were derived from the most sensitive assays identified so far, they likely represent worst-case estimates. In addition, methods that inactivate the biological activities of DNA can be assessed and estimations of risk reduction by these treatments can be made. In this paper, we discuss our approaches to address potential safety issues associated with residual cellular DNA from neoplastic cell substrates in viral vaccines, summarize the development of assays to quantify the oncogenic and infectivity activities of DNA, and discuss methods to reduce the biological activities of DNA.

Citation kindly provided by David Weisman

EMA documentation- Page 74



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Dr Ah Kahn Syed Writes Arkmedic's blog Mar 29

Great work again Kevin. The TGA defined limit of 10ng per dose is equivalent to the 1:3000 EMA limit for the 30microgram dose in COMIRNATY.

Document here

https://www.tga.gov.au/sites/default/files/pm-argpm-guidance-18.pdf

We will need to look at TGA FOI 3471 documents to see if they performed a quantitative assessment of DNA content.

If you take out the vial 3 RNA data as an outlier (SD>1) I get an overall (geometric mean) Ct for the RNA as 13.6 and for the DNA as 20.2...

Giving 94:1 overall RNA:DNA - 30x times the limit.

This is not as bad than the bivalent. I wonder if they just gave up any attempt to purify for the bivalent because they knew nobody cared?

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Weihan Xing Mar 29

WHY and HOW are these frankensteinian concoctions still on the market and being used?!

Governments everywhere have never before engaged in anything so criminally insane as mandating these poisons.

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2 replies

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