We the People 50-Recall the Shots Initiative

www.wethepeople50.com

www.formerfedsgroup.org

Contact:

Director, Scientific Liaison

Dr. Janci C. Lindsay, PhD.

Director of Toxicology & Molecular Biology

Toxicology Support Services, LLC.

Phone: (832) 646-1378

jlindsay@toxicologysupport.com

Project Coordinator, Legal Liaison

Carolyn Blakeman

Former Feds Group

Phone: (713) 540-6332

Carolyn.blakeman@formerfedsgroup.org

Media Contact

Kelley Collins

Declare Truth

(512) 701-6150

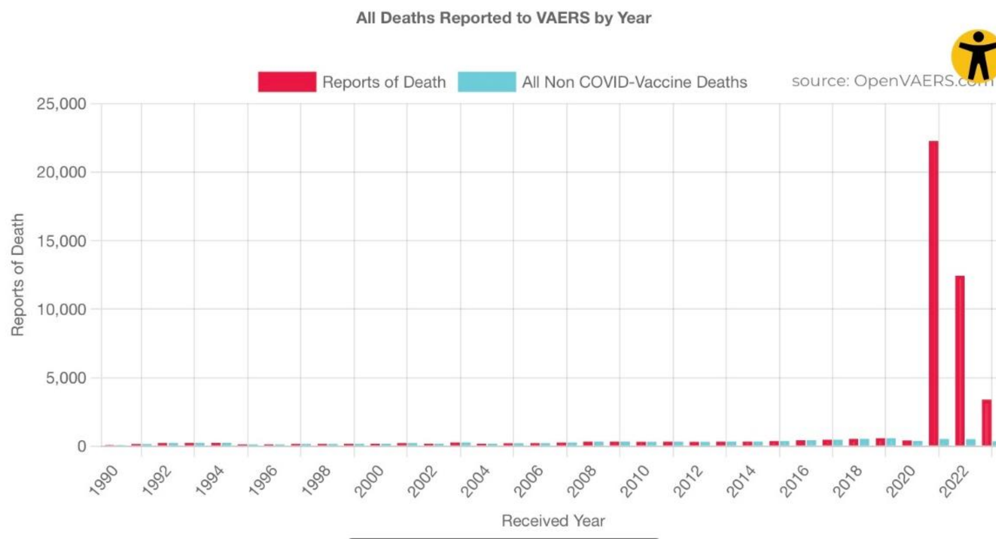
KelleyLCollins@proton.me

We the People 50-Recall the Shots

A FormerFedsGroup Freedom Initiative

Who We Are
The "We the people 50-Recall the Shots Initiative", are a group of doctors, scientists, healthcare workers, COVID-vaccine-injured, attorneys, concerned citizens, and pharma regulatory specialists, who have gathered together to demand that the COVID genetic vaccines as well as the entire genetic vaccine platform technology, be seized and recalled until further safety and formulation investigations, can be conducted.

Deaths Pile up as Regulatory Bodies Do Nothing to Recall the Shots
We have been highly alarmed at the lack of CDC, FDA, or manufacturer actions to pull the COVID genetic vaccines based upon the large number of adverse events and deaths reported, temporally associated with these shots. In the past, just 26 deaths prompted the recall of the swine flu vaccine. We are now in excess of 36,700 deaths in the CDC Vaccine Adverse Event Reporting System (VAERS) overall and 18,382 deaths attributed to US reports as of November 3, 2023—that is 700 times the number of deaths that previously prompted the Swine Flu vaccine recall. Pfizer's own documents show that even they recorded 1223 deaths within the first 90 days following the vaccine rollout, but there has been no recall. This is very unusual and questions regarding this inconsistency have been met with no logical response from either entity. The number of deaths alone reported into the VAERS system for the COVID shots dwarf all other traditional vaccines given for the past 30 years, *combined* (***See CDC VAERS Chart Below through November 3, 2023***).



COVID Vaccine Deaths are not a Function of More Doses Given, the Shots are More Lethal per Dose

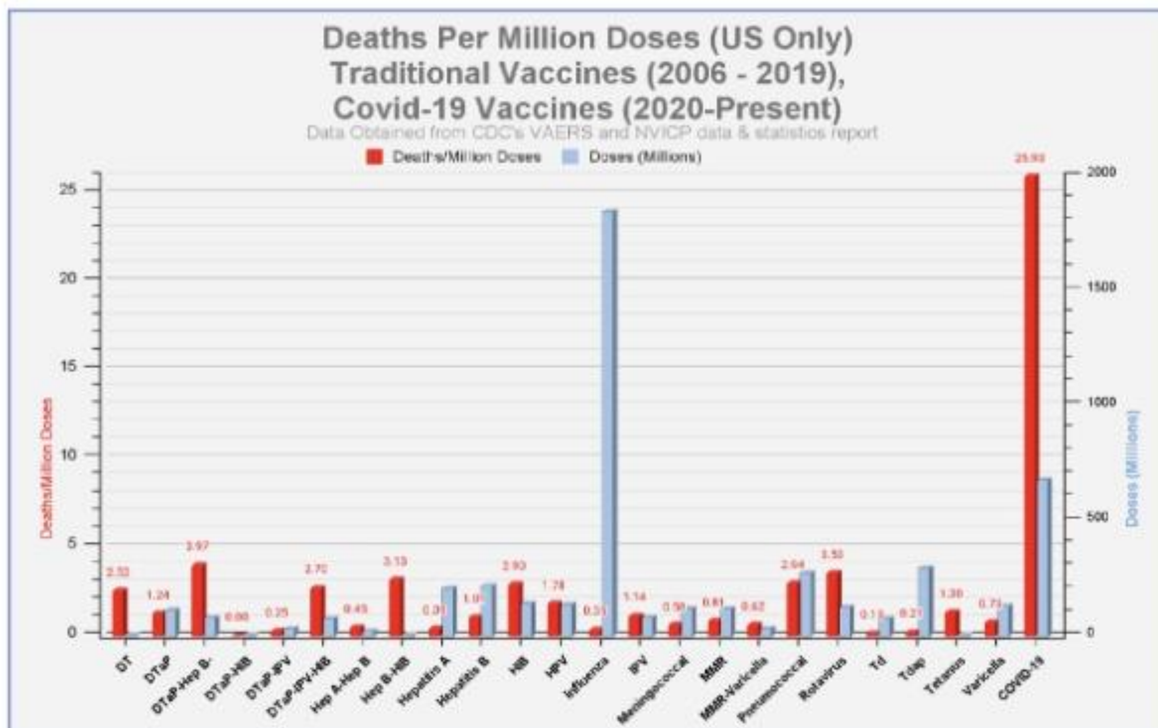


Fig. 4. : Rate of death reports per million doses associated with vaccines to the US CDC's VAERS pharmacovigilance database from 2006 to 11 August 2023. Source: VAERS Analysis: https://vaersanalysis.info/2023/08/18/vaers-summary-for-covid-19-vaccines-through-8-11-2023/.

COVID vaccine deaths not a matter of more doses given as is popularly argued. In the graph above the doses are given in light blue and the deaths per million in red. It is very clear to see that the COVID genetic shots are more lethal per dose at almost 26 deaths per million doses compared to the Influenza vaccine at 0.35 deaths per million doses. This despite the fact that the Influenza vaccine has been given to millions more people.

Why we are Demanding a Recall of the COVID Genetic Vaccines and a Halt on the Technology

These genetic vaccines must be immediately recalled and investigated due to a number of serious issues around their composition and manufacture, including contamination and adulteration, failure to adhere to cGMP and unacceptable batch to batch variation, frameshifting leading to additional protein products outside of the intended spike protein, as well as misrepresentations as to safety and efficacy and frank mislabeling and misbranding of the products.

Recent investigations by several different researchers have found there to be significant contamination of the mRNA shots with the E.Coli DNA plasmids used to

make the shots in large volume. Some of the shots, (Pfizer) are adulterated with non-disclosed genetic sequences from a virus known to be cancer causing in humans—SV40. The mRNA is also heavily broken and degraded and contaminated with bacterial endotoxin.

DNA plasmid contamination, adulteration with SV40 sequences, contamination with bacterial endotoxin and degradation of the mRNA genetic sequence, can lead to genomic integration, gene silencing, severe adverse immune events, anaphylaxis, all types of cancers and death—all of which have shown temporal association to administration of these genetic vaccines.

It has also very recently been verified that early evidence of frame-shift mutations of the genetic sequence in translation, is leading to multiple aberrant peptide and proteins. In plain language, instead of causing the production of just the viral spike protein, the genetic sequences are being mistranslated into other proteins against which the body is also mounting an immune response. This is a fatal flaw to the technology which is extremely dangerous and which cannot be fixed.

These are additional reasons the entire genetic vaccine platform itself must be pulled along with the COVID genetic vaccines: 1) In order to scale up to the amount of shots necessary to give to the population, bacterial expression systems must be deployed and this technology cannot remove dangerous genetic sequence contaminants using current methodology. 2) This mRNA approach will always lead to frame to frame shift mutations which will cause for any variety of mutated proteins to be made which is dangerous and even lethal. 3) This type of approach—whether mRNA or DNA, will always cause an immune reaction to be targeted against one's own cells which display the viral or bacterial foreign antigen, and any other antigen created by frame shift mutations, creating a condition for auto-immunity, cancers, tolerance—or all.

Contamination and Adulteration as well as Misrepresentation and Misbranding Violate State Consumer Product Protection Laws

It is not appropriate to allow contaminated and adulterated medical products to remain on the market where they can be injected into people, especially into our infants and children. There are an egregious number of adverse events and deaths that have been reported into the CDC VAERSs and V-SAFE systems, other databases, and to the manufacturers, which show clear evidence of harm and lethality as compared to all other traditional vaccines for the past 30 years.

The mRNA shots were also misrepresented as being identical by manufacturing process to the shots that were given in the clinical trials, when they are not. Two completely different processes were used which resulted in differing formulations.

Process 1 did not use bacterial plasmid template and was used in the clinical trials and process 2 did use bacterial plasmid template and was used on the population. Contamination and adulteration of consumer products and misrepresentations of formulations of manufacturing process and clinical trial safety data, violates the Consumer Product Protection statutes of most states.

Good Manufacturing Practice Violations are Ignored for These Products

Investigations by pharma process engineers and pharma regulatory specialists, have revealed poor manufacturing practices and failure to adhere to cGMP, coupled with a lack of proper regulatory oversight by the FDA and the manufacturers, of lot-to-lot purity and consistency in the manufacturing process.

The lot-to-lot variability in adverse events and deaths, supports the laboratory findings of contamination and degradation variance between identical lots of product, which should not be present had they undergone proper safety screening prior to release. The FDA has failed to seize the contaminated, adulterated and degraded COVID vaccines and investigate these findings despite being made aware of them, several months to years ago. Additionally, there has been inadequate and dishonest responses from the FDA, the CDC and the manufacturers themselves on these findings, prompting this action by We the People 50-Recall the Shots, to go to the States directly and demand their removal and an immediate investigation into these issues, for the safety of the citizens involved.

Regulatory framework around these COVID genetic vaccines allows for them to be misbranded and adulterated and not subject to cGMP while still being "approved" and "licensed" by the FDA

Recent information on how these shots are most likely regulated, as "medical countermeasures" and "medical products" under a Public Health Emergency (PHE) and with PREP act liability protections is also quite alarming, literally allowing for the specific "**adulteration and misbranding**" of these products which are also under these conditions ordered to be approved and licensed—even in the absence of cGMP, which is not required for these medical countermeasures (see 21 CFR 360 bbb-3a):

"(3) Effect

Notwithstanding any other provision of this chapter or the Public Health Service Act [42 U.S.C. 201 et seq.], an eligible product shall not be considered an unapproved product (as defined in section 360bbb–3(a)(2)(A) of this title) and shall not be deemed adulterated or misbranded under this chapter because, with respect to such product, the Secretary has, under paragraph (1), extended the expiration date and authorized the introduction or delivery for introduction into interstate commerce of such product after the expiration date provided by the manufacturer.

"(4) Expiration date

For purposes of this subsection, the term "expiration date" means the date established through appropriate stability testing required by the regulations issued by the Secretary to ensure that the product meets applicable standards of identity, strength, quality, and purity at the time of use.

(c) Current good manufacturing practice

(1) In general

The Secretary may, when the circumstances of a domestic, military, or public health emergency or material threat described in subsection (a)(1)(C) so warrant, authorize, with respect to an eligible product, deviations from current good manufacturing practice requirements otherwise applicable to the manufacture, processing, packing, or holding of products subject to regulation under this chapter, including requirements under section 351 or 360j(f)(1) of this title or applicable conditions prescribed with respect to the eligible product by an order under section 360j(f)(2) of this title.

(2) Effect

Notwithstanding any other provision of this chapter or the Public Health Service Act [42 U.S.C. 201 et seq.], an eligible product shall not be considered an unapproved product (as defined in section 360bbb–3(a)(2)(A) of this title) and shall not be deemed adulterated or misbranded under this chapter because, with respect to such product, the Secretary has authorized deviations from current good manufacturing practices under paragraph (1)." [1]

There are several additional points which we wish to bring to your attention:

1. The genetic vaccines are what has traditionally been termed "gene therapy" for regulatory purposes or "genetic biologics"—the administration of a genetic sequence that encodes the protein that the "transfected" cells, will then produce.[2] In the early days gene therapy was used in "right to try" cases in order to correct lethal genetic defects in essential proteins. In more recent times the technology has been explored as a way to make internal "vaccines" as well as augment human physiology with desired traits.

2. This technology, gene therapy, has been researched over the past 4 decades, but was never brought to market due to the severe adverse events that were seen in the early trials, which included lethal auto-immune reactions and latent cancers, that emerged consistently several years after the administration of the gene product. The cancers were thought to be due to the given gene integrating into the genome and causing the expression of mutated proteins. The lethal autoimmune reactions were thought to be due to the attempt to express a foreign or modified protein on "self" cells. Twenty to thirty years ago the greatest concerns surrounding the large-scale use of gene therapies, were cancers and "accidental" gene transfer, should the therapies make it to the testes or ovaries and

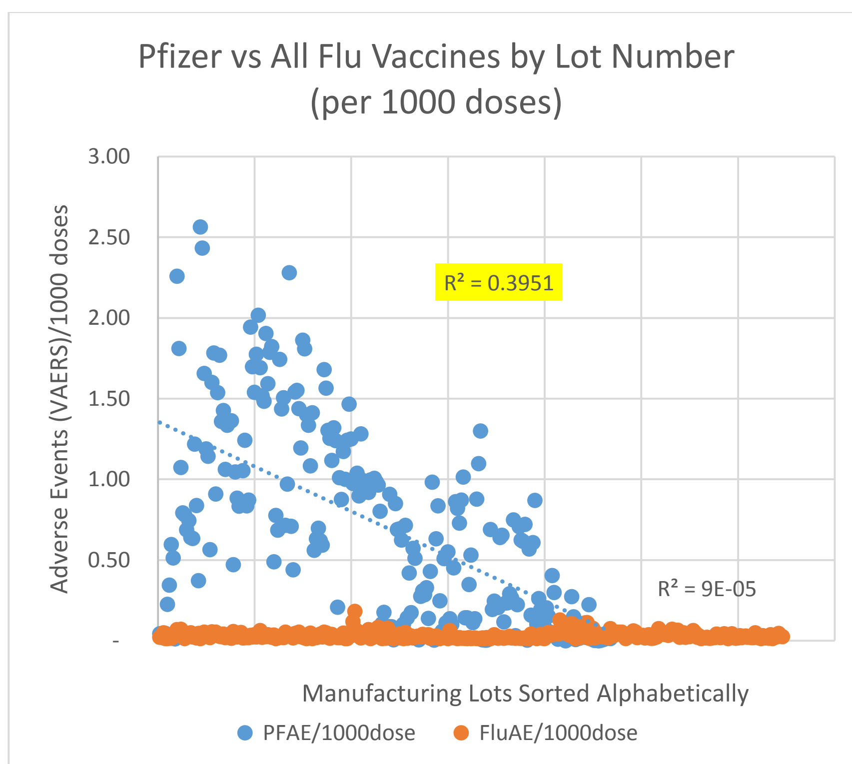
[1] 21 USC 360bbb-4b: Medical countermeasure master files (house.gov)

[2] Banoun H. mRNA: Vaccine or Gene Therapy? The Safety Regulatory Issues. Int J Mol Sci. 2023 Jun 22;24(13):10514. doi: 10.3390/ijms241310514. PMID: 37445690; PMCID: PMC10342157.

contamination of the gene pool. Recipients were often sterilized prior to receiving the technology to avoid inadvertently passing on the administered gene.[3] Therefore it was quite surprising that this technology was rolled out en-masse recently without close monitoring of any of these conditions. Since their rollout we have learned that the mRNA in the lipid nanoparticles make it to both the testes and the ovaries where they are directing the expression of the spike protein.

3. There has been a wide variability to the deaths and disability of the COVID vaccines by lots, which was very different than the typical lot to lot consistency of AE's that you might see with flu lots (***see chart below included in attached witness statement of pharma regulatory specialist Sasha Latypova Attachment A)***. This prompted much concern amongst scientists and regulatory experts, early on, who knew that with typical regulatory oversight, these lots should have been flagged right away and examined for contamination or degradation. Degradation of the mRNA in the shots was noted by Europe's equivalent to the FDA, the EMA, in early license applications by the manufacturers in 2020 and 2021.

Pfizer COVID Genetic Vaccine Adverse Events by Lot Compared to Flu Vaccine



[3] Nancy M. P. King. "Accident & Desire: Inadvertent Germline Effects in Clinical Research." *The Hastings Center Report*, vol. 33, no. 2, 2003, pp. 23–30. *JSTOR*, https://doi.org/10.2307/3528151.

4. Further investigations showed the continued presence of mRNA degradation of the COVID shots. Degradation of the mRNA could cause the production of mutated proteins or silence protein expression. This is also very dangerous. There was apparently no oversight to ensure that this problem was corrected before administering these shots to the public and vulnerable infants and children as well as pregnant women.

5. It has also been found that the process to make the make the shots in E.Coli bacteria using bio-engineered DNA plasmids (process 2) was not the process used to make the majority of the shots administered in the clinical trials (Process 1). Only 242 subjects out of over 40,000 subjects received the process 2 plasmid derived shots in the clinical trials. The rest of the clinical trial subjects received the synthesized shots that were not contaminated with the DNA self-replicating plasmids. This was kept from the public and they were led to believe that what was tested in the clinical trials was what they were receiving in their arms.

6. These are the statements from an affidavit by Pharma specialist Sasha Latypova which speak to this evidence of degraded and contaminated COVID vaccine product. ***(See attached full witness statement by Sasha Latypova)***:

   a. **"The modified RNA (mRNA) which is the active substance of Pfizer's vaccine BNT162b2 is allowed to vary in its integrity by up to 50% in the finished product.**

   b. **Product impurities in the form of truncated mRNA, untranslated DNA and other unknown nucleic acid constructs have been allowed in the finished product in unspecified quantities.**

   c. **As a result of the reckless widening of quality acceptance criteria for the integrity of active ingredient in manufacturing batches, there is a great variation in resulting formulations of final product as dispensed in vials. Furthermore, the contents of the vials are cut by hand into multiple doses by untrained and unsupervised vaccinators who are working outside of the Good Manufacturing Practice compliance.**

   d. **There is an excessive variation in the rates of adverse events and deaths observed post-vaccination for different manufacturing batches which far exceeds expected batch-to-batch variations for compendia pharmaceutical products, such as for example seasonal flu vaccines."**

7. Given the past experience with this technology, and its first time use in massive amounts of the population, you would expect that the regulatory

oversight for these shots, would be all that more stringent to ensure the safety of the new technology. Unfortunately, this did not happen. Both Pfizer and Moderna contracted out the manufacture of these shots to other companies including Lonza, Renschler and Cataland. These companies received FDA 483 forms citing their multiple violations of good manufacturing practice at their facilities, cGMP. There is no indication that these deficits were corrected, and there are no follow up to the original citations issued, but they were all allowed to continue to produce the shots that were administered without pause to the world. To our knowledge, not a single lot has been pulled from the market, despite a strong and variable lot-to-lot association with AE's and death (***See attached declaration of Pharma Process and Regulatory Specialist, Hedley Reese, Attachment B, and links below***).

- ***Catalent's Belgium operations get a second FDA scolding within 1-year span***
- ***Catalent cuts 2023 sales expectations as productivity issues and costs pile up at 3 plants***
- ***Rentschler slapped with FDA Form 483 citing lax manufacturing procedures***
- ***Moderna's new booster launch tripped up by production issues at Catalent plant***
- ***BioNTech gets rolling with mRNA production at former Novartis site in Marburg***

8. It has now been confirmed by several laboratories as well as the FDA equivalent in Canada—Health Canada, that both the Pfizer and Moderna mRNA monovalent and bivalent (booster) vaccines are contaminated with the DNA plasmids that are used to create the shots on a large scale. ***See the following publications as well as attachments C:***

- **Kevin P. McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin. 2023 Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose. 10.31219/osf.io/b9t7m 10.31219/osf.io/b9t7m**

- **Speicher, D. J., Rose, J., Gutschi, L. M., Wiseman, D. M., PhD, & McKernan, K. (2023, October 19). DNA fragments detected in monovalent and bivalent Pfizer/BioNTech and Moderna modRNA COVID-19 vaccines from Ontario, Canada: Exploratory dose response relationship with serious adverse events. https://doi.org/10.31219/osf.io/mjc97**

- **EXCLUSIVE: Health Canada Confirms Undisclosed Presence of DNA Sequence in Pfizer Shot | The Epoch Times**

- **COVID-19 mRNA vaccines contain excessive quantities of bacterial DNA: evidence and implications. https://doctors4covidethics.org/covid-19-mrna-vaccines-contain-excessive-quantities-of-bacterial-dna-evidence-and-implications/**

9. The plasmid DNA is supposed to be purified away from the mRNA final product before administration, as residual DNA in significant amounts or any kind is carefully regulated in vaccines due to the dangers of its presence and potential consequences. In the case of plasmid DNA, no amount of residual plasmid DNA is acceptable especially in its transfective form, as it can replicate in the body in gut E. Coli once administered. One of the dangers of the plasmids in the shots is the potential for these plasmids, if intact, to infect (transfect) the E.Coli in the gut of the recipient, making them a continual spike protein factory. This may explain the detection of the spike protein in the brain up to 9 months out from injection. as well as the phenomenon of "Long Covid" in the vaccinated, as well as explain the excess deaths and adverse events, in part.

10. The plasmids contain non-disclosed sequences for an SV40 promoter with an SV40 nuclear localization signal, from a virus that is known to cause cancer. These sequences which allow the DNA plasmids to transfect human cells, should not be there and were hidden from regulatory bodies in the plasmid maps submitted. These sequences are not necessary if the plasmids are only to be used to make mass amounts of the shot material. This SV40 sequence can also bind to a tumor suppressor protein called P53 which inactivates it for its intended purpose. The encoded vaccine spike protein also binds to P53 with the same predicted effect.

11. The plasmids also contain an antibiotic resistance gene cassette for Kanamycin and Neomycin, which are antibiotic classes widely used in medicine to treat bacterial infections such as tuberculosis. Recipients of these shots could become resistant to treatment with these classes of antibiotics creating a public health emergency of unimaginable consequence. The presence of these plasmids in significant amounts in every vial tested, is a grave concern which demands immediate seizure and recall of these products!

12. The spike protein was not meant to be continually produced. Moreover, the continued production of the DNA encoding for the spike in the body will increase the chance of genomic integration and cancers. These could also be passed on more easily through the gametes, though intercourse, breast milk and contact with others through the well-known mechanism of "shedding". Shedding studies of all gene therapies is recommended by the

FDA, but was never conducted by the manufacturers on these genetic vaccines. Nor, was shedding monitored in the public after the COVID vaccines' large scale release, despite thousands of reports of adverse events including vaginal bleeding, miscarriage and even stroke following the unvaccinated being in close proximity to the recently vaccinated.[4]

13. Additional concerns regarding the DNA plasmid contamination is the possibility of the concurrent contamination with **E. Coli** bacterial proteins and "endotoxin", LPS. These, if present, would cause massive immune reactions and sepsis in the recipients. It is plausible that this contamination exists given the shoddy manufacturing practices, the presence of the DNA plasmids and the fact that these shots were grown in **E. Coli**. [5]

14. It has also just recently been proven although it was seen very early on through proteomics studies, that the COVID mRNA vaccines are producing off-target proteins and pieces of proteins of unknown and varying identity, due to frame shift mutations that is occurring during translation within the cellular machinery. This is alarming and very dangerous as it is a cancer risk as well as poses other serious health risks and it is yet another reason to immediately pull these shots as we have no idea what proteins and pieces of proteins are being produced as a result of these gene therapies and we CANNOT continue to inject these into anyone.[6,7]

Had these contaminations and adulterations been noted in infant formula or even dog food, these products would have been immediately seized and recalled without even a single death, none the less over 36,700 deaths—many in previously healthy children.[8] These are products that we are allowing, in fact even

---

[4] Banoun H. Current state of knowledge on the excretion of mRNA and spike produced by anti-COVID-19 mRNA vaccines; possibility of contamination of the entourage of those vaccinated by these products. *Infect Dis Res*. 2022;3(4):22. doi:10.53388/IDR20221125022

[5] Schmeling, M, Manniche, V, Hansen, PR. Batch-dependent safety of the BNT162b2 mRNA COVID-19 vaccine. Eur J Clin Invest. 2023; 00:e13998. doi:10.1111/eci.13998. https://onlinelibrary.wiley.com/doi/10.1111/eci.13998

[6] Mulroney, T.E., Pöyry, T., Yam-Puc, J.C. *et al.* N^1-methylpseudouridylation of mRNA causes +1 ribosomal frameshifting. *Nature* (2023). https://doi.org/10.1038/s41586-023-06800-3

[7] Wiseman, David. (2023). Ribosomal frameshifting and misreading of mRNA in COVID-19 vaccines produces "off-target" proteins and immune responses eliciting safety concerns: Comment on UK study by Mulroney et al. 10.13140/RG.2.2.36710.40005.

[8] www.openvaers.com/covid-data/child-summaries

mandating be injected into our infants and children in some cases, where we are recording heart attacks, strokes and "sudden deaths" during their sleep and no one has demanded their recall. This is a travesty. It is not enough to just give people a "choice" of whether to take these products or not as contaminated and adulterated medical products known to have lethal effects should never be a CHOICE for children. Children do not have a choice and these shots have been added to the childhood schedule which gives a false sense of security about their safety and efficacy. While these shots are not mandated for children, they are heavily incentivized and encouraged with some Pediatricians removing children from their practices who do not receive all the shots on the schedule including the COVID vaccines. Additionally, transplant recipients, including pediatric patients, are being denied lifesaving organ transplants by medical providers, for failure to take these contaminated shots.

Additional Guidance for Attorney Generals as to the Legal Framework which must be Acknowledged and Challenged

There is additional guidance for attorney generals which has been provided and is as follows that speak to the unconstitutional delegation of authority to the Health and Human Services (HHS) secretary and the purported legalization of distributing misbranded and adulterated medical products under a "Public Health Emergency", as well as unlawful protections offered to those who would knowingly commit fraudulent acts:

State Attorneys General should build on what has been learned through *Jackson v. Ventavia, Pfizer et al*; *Bridges v. Houston Methodist Hospital*, and *Texas, Oklahoma et al v. US Department of Health and Human Services, Xavier Becerra et al*, (4:23-cv-00066-Y) Federal complaints could be filed at the Supreme Court, under SCOTUS original jurisdiction on constitutional matters (US Constitution, Art III.S2.C2.2), to have the *Public Health Emergencies* sections of the Public Health Service Act (42 USC 247d through 42 USC 247d-12) and the *Expanded access to unapproved therapies and diagnostics* sections of the Food Drug and Cosmetics Act (21 USC 360bbb through 21 USC 360bbb-8d) **declared null and void *ab initio* (from the beginning)…**

This is because those laws were enacted unconstitutionally outside the power (*ultra vires*) of Congress and Presidents to draft and sign any laws that:

1. enable US government officials operating within the executive and administrative branches to plan and commit mass fraud using EUA

"countermeasure" vaccines and medicines under "public health emergency" decrees;
2. block the constitutional separation of powers authority of federal courts to review and halt such criminal acts by the federal executive branch [42 USC 247d-6d(b)(7)];
3. block the constitutional separation of powers authority of Congress to review and halt such criminal acts by the federal executive branch [42 USC 247d-6d(b)(9)];
4. block the constitutional (federalism) authority of state, tribal and local authorities to review and halt such criminal acts by the federal executive branch [42 USC 247d-6d(b)(8)];

The state AG litigation should challenge two key Congressional acts: the 2004 Project Bioshield Act, and the 2005 Public Readiness and Emergency Preparedness (PREP) Act. Without Congress enacting and US presidents signing those two laws, the mass fraud failure to recall these dangerous shots could not have happened.

Because of the corruption of law that those two Congressional acts in 2004 and 2005 — and their precedent and successor acts — have wrought, the entire PHSA (first enacted 1944) and FDCA (first enacted 1938) should also be nullified and all executive branch public health agencies and programs should be judicially and/or legislatively dismantled, as they have been turned into enterprises which incentivize profit and malfeasance to the public without any liability for harm.

We seek to use the Consumer Product Protection laws of most states to remove these products from the commerce stream for the egregious neglect of cGMP of these products which were mandated to many and encouraged for all, despite the knowledge of their un-safe, contaminated, adulterated and degraded nature. With these documents you now have full knowledge that these shots are being allowed to be distributed adulterated and misbranded without any cGMP oversight while the FDA and CDC are telling the public that they are “safe and effective”. Contaminated, adulterated, misrepresented and misbranded products that do not undergo good manufacturing practice, cannot, on their face, be “safe”. We have also learned that these products were never tested for efficacy to stop the contraction or transmission of infection, as admitted by Pfizer

and FDA.[9] Please move to protect your citizens and recall these dangerous shots while also banning the use of the dangerous genetic vaccine technology in your state.

We hope that these materials help inform of our concerns and for the undeniable necessity to recall these dangerous genetic vaccines. We hope that you will take legal action to reform the laws that have allowed these shots to remain in the product stream despite their clear lack of safety and efficacy. This must be done despite unlawful protections afforded to the manufacturers.

We the People 50-Recall the Shots, Committee— January, 2023

[9] Kingwell K. COVID vaccines: "We flew the aeroplane while we were still building it". Nature Reviews Drug Disc 2022. Epub Nov 11 http://doi.org/doi.org/10.1038/d41573-022-00191-2

Attachment A

Declaration of Pharma Specialist, Sasha Latypova

Excessive Variability in Pfizer's BNT162 Vaccine Formulation Batch-to-Batch

My Background and Experience:

I am a retired business executive with 20+ years of experience in pharmaceutical and medical device Research and Development (R&D) industry as well as in a broader data analytics field. Throughout my career my primary expertise was innovation in technologies used in drug development, as well as collection and analysis of data from global clinical trials. My experience covers all therapeutic areas of drug development. I was senior executive at several clinical research organizations (CROs) conducting data collection and analysis on behalf of pharmaceutical companies for the purpose of clinical trial data submissions to regulatory authorities such as FDA, EMA and other relevant government agencies. I have extensive experience working with the FDA staff on issues related to safety assessments of novel pharmaceuticals. Prior to working in the CRO field, I worked as analytical consultant in econometrics and litigation support, working primarily for pharmaceutical and medical device clients. I hold Master of Business Administration degree from Dartmouth College, Hanover, NH.

The following statements are based on my review of documentation that has been publicly disclosed from Pfizer, European Medicines Agency (EMA) and Food and Drug Administration (FDA) and relates to the Chemistry, Manufacturing and Controls (CMC) sections of Pfizer's BNT162 dossier. The documents were released due to a cyberattack on the EMA (see Attachment). The EMA acknowledged the release of the documents and did not dispute their authenticity. Furthermore, the British Medical Journal confirmed the contents of these documents with respect to the issues of integrity of the active ingredient discussed herein through correspondence with the EMA, MHRA, FDA, Health Canada and Pfizer.[1]

The rates of adverse events and deaths per manufacturing batch number are derived from CDC VAERS database.

My affidavit attests to the following facts identified in the documents, with evidence information provided below:

1. The modified RNA (mRNA) which is the active substance of Pfizer's vaccine BNT162b2 is allowed to vary in its integrity by up to 50% in the finished product.
2. Product impurities in the form of truncated mRNA, untranslated DNA and other unknown nucleic acid constructs have been allowed in the finished product in unspecified quantities.
3. As a result of the reckless widening of quality acceptance criteria for the integrity of active ingredient in manufacturing batches, there is a great variation in resulting formulations of final product as dispensed in vials. Furthermore, the contents of the vials are cut by hand into multiple doses by untrained and

[1] https://www.bmj.com/content/372/bmj.n627

unsupervised vaccinators who are working outside of the Good Manufacturing Practice compliance.

4. There is an excessive variation in the rates of adverse events and deaths observed post-vaccination for different manufacturing batches which far exceeds expected batch-to-batch variations for compendial pharmaceutical products, such as for example seasonal flu vaccines.

Evidence from EMA and Pfizer Documents:

Lack of mRNA integrity and product impurities (fragmented nucleic acid chains) were found in Pfizer's product days before it was authorized for market:

mRNA integrity, and conversely, its instability, is one of the most important variables relevant to all mRNA vaccines. Pfizer and BioNTech repeatedly stated that the efficacy of the product is highly dependent on the quantity of the sufficiently intact mRNA molecule. Even a minor degradation reaction, anywhere along a mRNA strand, can severely slow or stop proper translation performance of that strand and thus result in the incomplete expression of the target antigen.

Pfizer made several major changes to its manufacturing process going from small clinical scale manufacturing (Process 1) to commercial scale (Process 2) as described in the "Rapporteurs Rolling Review Report", p. 57 (full document in Attachment).

"Process 1

[…]two changes were made within Process 1 between nonclinical toxicology and Phase 1/2/3 process: the scale of the reaction and the site. The increase in scale was required to make sufficient material for clinical trials. The location changed from a non-GMP lab into GMP facilities. This process was based on BioNTech platform knowledge from other mRNA therapeutic programs.

Process 2

[…]The DNA template changed from a PCR template to linearized plasmid DNA in order to meet commercial demands. Additionally, the magnetic bead purification was replaced with proteinase K digestion and UFDF steps. The magnetic bead purification method was not scalable, but removed small molecule impurities (e.g. spermidine, DTT), residual DNA, and enzyme impurities (e.g. T7 polymerase, DNase I). […]"

These changes were performed without re-validation of the manufacturing process or re-running the preclinical and clinical studies to confirm comparability on safety and efficacy characteristics of the product. Importantly, these changes resulted in a substantial drop in the integrity of key active ingredient – mRNA molecule as measured by the %mRNA integrity and % of fragments (Late Migrating Species, LMC) in each manufactured batch. This was identified by the regulatory reviewers at EMA and FDA, and EMA specifically recorded this as a Major Objection #2, i.e. a regulatory flag that

required a resolution prior to the product approval. The discussions around this issue are recorded in numerous documents that were released from EMA, at the end of November 2020, including email exchanges between EMA staff and management (see Emails in Attachment). For example, a PowerPoint document from the meeting on November 26, 2020 between EMA and Pfizer/BioNTech describes the issue of mRNA integrity (see 20201126_BNT162b2_EMAmeeting14.pdf in Attachment).

In this meeting it was discussed that the batches manufactured with Process 2 had a much lower range of % intact mRNA and higher % of impurities – fragmented nucleic acid chains of various length and type (DNA and RNA). Specifically, p. 20 lists final product batches manufactured with both processes, ranging in mRNA integrity from 55% to 85% with the remaining % of volume occupied by uncharacterized fragments.

EMA regulatory concern with lack of mRNA integrity in Pfizer's product was evident. Specifically, on p. 4 the document states that:

"Significant differences between batches manufactured by DS Process 1 and 2 are observed for the CQA *[critical quality attribute]* mRNA integrity. In addition, the characterisation of BNT162b2 DS *[drug substance]* is currently not found acceptable in relation to this quality attribute. This is especially important considering that the current DS and DP *[drug product]* acceptance criteria allows (sic) for up to 50% fragmented species."

Further, on p. 5 the reviewers discussed the presence of uncharacterized fragmented nucleic chains, some long enough to translate into unknown proteins, and deemed them product impurities that required further characterization:

"Truncated and modified RNA species should be regarded as product-related impurities. Even though two methods, namely agarose gel electrophoresis and capillary gel electrophoresis (CGE), have been applied to determine RNA integrity of BNT162b2 DS *[drug substance]*, no characterisation (sic) data on truncated forms is presented. "

As a result of the manufacturing inconsistency, the clinical trial data collected using the Process 1 material was not deemed applicable to the material manufactured in Process 2. Several EMA reviewers wanted to understand the potential impact on safety and efficacy via bridging clinical studies (see Emails in Attachment). No such comparisons were done. Pfizer provided comparison of some chemical analyses from various batches, but no further characterization of the fragments of RNA and DNA or study of impact of these impurities on safety and efficacy of patients was provided.

EMA reviewers and Pfizer "resolved" this Major Objection by arbitrarily lowering the acceptance criteria for %mRNA integrity (see p.4):

"In addition, we are revising the RNA integrity specification for drug substance to >=60%, drug product release to >=55%, and drug product shelf life to >=50%. "

An extremely wide variation of the integrity of the active substance in bulk material (batch) of the product and abundant presence of uncharacterized impurities means that batches of different formulation - and thus different potency and safety profiles - are being produced. This variation is further amplified when the bulk material is filled in small quantities into vials. Each batch of Pfizer product contains approximately 300,000 vials filled with 0.45ml of drug product which may get varying quantities of intact and broken mRNA molecules. In addition, at the final step of administration, this variability is further exacerbated by dose preparation in a non-GMP environment by untrained and unsupervised staff at the vaccination centers.

Both the regulators and Pfizer to date have not disclosed the acceptable ranges for the key ingredients of the vaccine product, neither in bulk product nor in a vial (as dispensed), and claim “commercial secrets” that prevent them from doing so.

Evidence from adverse event reports (in VAERS database) analyzed by manufacturing lot number.

Manufacturing of pharmaceutical products is regulated by laws that are established to control within tight ranges acceptable criteria for the identity, quantity, quality, purity, potency and other characteristics of the product ingredients to ensure safety and conformity to the approved product labeling. It is expected that the product lot-to-lot, or batch-to-batch, is essentially the same. Therefore, when outcomes data such as rates of adverse events reported for each manufacturing lot is examined, it is expected that only minor variations from lot-to-lot may be observed. This is true for conventional pharmaceutical products and for traditional vaccines such as seasonal flu vaccines.

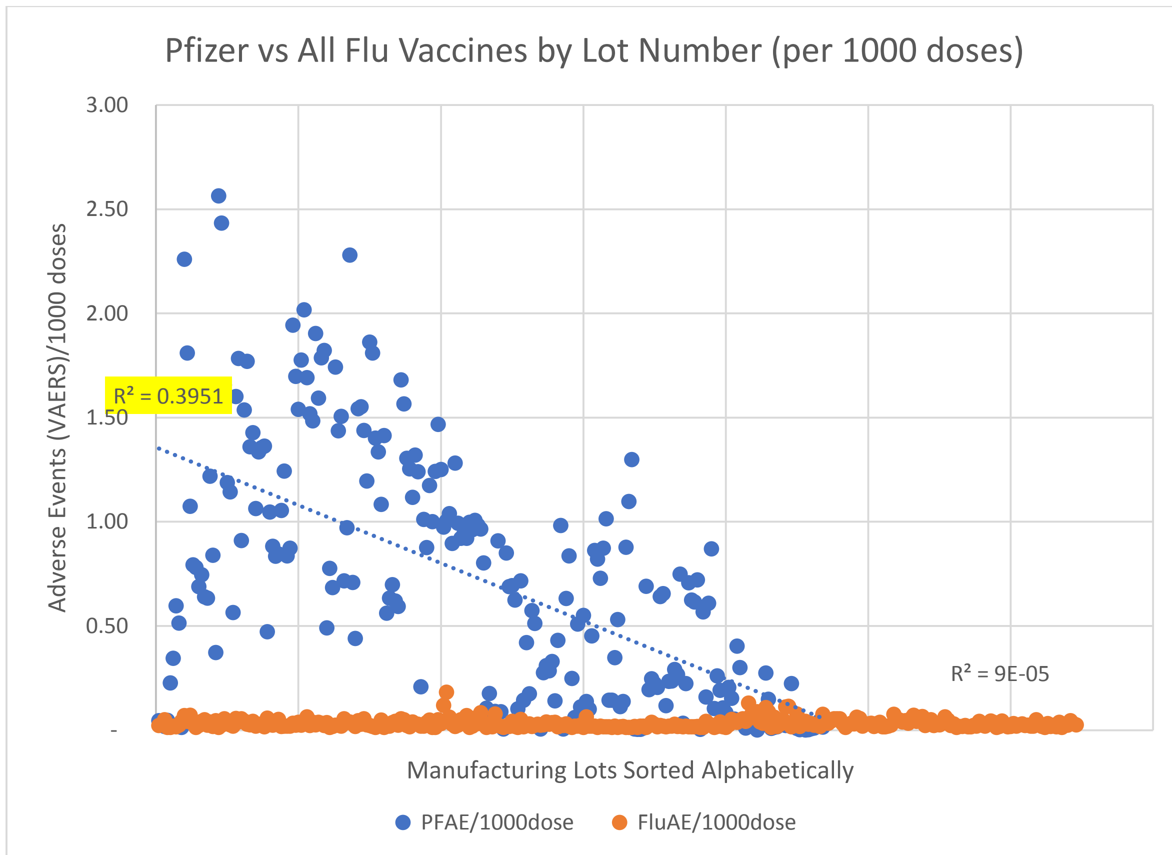
There is an excessive variation in the rates of adverse events and deaths observed post-vaccination for different manufacturing batches which far exceeds expected batch-to-batch variations for compendial pharmaceutical products, such as for example seasonal flu vaccines.

The graph below shows a comparison between the manufacturing lots of Pfizer’s BNT162b2 product and manufacturing lots of all seasonal flu vaccines released in 2019-2020. The lot numbers for Pfizer were verified with CDC and dates of manufacture and expiration were obtained. The flu vaccine lot numbers were obtained by downloading data from VAERS. Rates of adverse events reported for each lot are plotted against the lot number (not shown on X-axis for clarity), sorted alphabetically. Finally, the adverse event rates are expressed in “per 1000 doses” to normalize for the lot size.

As evident from this analysis, there is an excessive variability in the toxicity (rates of adverse events) for Pfizer product. The flu vaccine lots in comparison look very similar to each other and have overall a very low rate of adverse events. There is a large correlation between the adverse even rates for Pfizer lots with the lot number (R^2=0.4). This should not happen. There should be no difference in the safety (toxicity) of a

product depending on how its manufacturing lot is numbered. This does not exist for the flu vaccine lot numbers. Overall, the rate of adverse events per lot/dose adjusted is extremely high as can be visualized on the graph below.

The difference between the two sets of products is stark and cannot be explained by normal demographic variations such as age or underlying health status of the recipient. Flu vaccines are administered to approximately 50% of population, including to old and frail people with compromised health status as well.



In conclusion, the evidence presented in my statement shows that Pfizer's manufacturing quality acceptance criteria permit for an extremely large variation of the key ingredient (up to 50%) and allow for a substantial presence of uncharacterized impurities. This can be deemed as product adulteration with de-facto different formulations produced in different batches. This leads to overall large rates of toxicities, reported adverse events and to extreme variations of product safety (toxicity) parameters in different manufactured lots.

Alexandra (Sasha) Latypova

PO Box 2981

Stateline, NV, 89449, USA

latypova@hotmail.com

Attachment B

Declaration of Pharma Specialist Hedley Rees

DECLARATION OF HEDLEY REES, B. ENG., HONS., EXECUTIVE MBA.

Pursuant to 28 U.S.C. § 1746, Hedley Rees, Bridgend, United Kingdom, hereby declares:

I am over the age of 18 and fully competent to make this declaration through my education, knowledge, experience, and training, of the facts stated in this declaration.

This declaration is submitted in support of: LEGAL ACTIONS TO CONVENE A GRAND JURY AND TO PULL THE COVID-19 "VACCINES" UNDER CONSUMER PRODUCT PROTECTION STATUTES FOR LACK OF SAFETY AND EFFICACY, MISREPRESENTATION, MISBRANDING, ADULTERATION AND DEGRADATION, CAUSES.

DECLARATION FOLLOWS:

Based on my experience, knowledge, and training as a pharmaceutical and biologics supply chain management and regulatory specialist (CV here)[i], it is my professional opinion that the companies with the responsibility to develop, manufacture, and distribute the SARS-CoV-2 injections, engaged in gross deviations from the Code of Federal Regulations, Title 21 (FDA regulations), as enacted under the Federal Food, Drug and Cosmetics Act.

Additionally, it is my opinion that the US FDA failed to fulfil its own obligations in ensuring that the companies above had sufficient knowledge, skills, experience, and capability to assure integrity of their supply chains. For example, it is unprecedented for FDA to approve an NDA or BLA without physical pre-approval inspections (PAIs) carried out by FDA on drug substance (DS) and drug product (DP) manufacturers, as a minimum.

Under the circumstances above, the safety and efficacy of the SARS-CoV-2 injections is

reasonably called into question, and so is the expected incidence of defective products produced due to regulatory non-compliances. The prudent action would be to immediately halt all activity involved in the development, manufacture and distribution of SARS-CoV-2 injections, and any other gene-based therapies, while suitable investigations are undertaken.

1. SARS-CoV-2 injections are categorized by FDA as Advanced Therapies, which include gene-therapies as therapeutic vaccines and other antigen-specific active immunotherapies.[ii]

2. Advanced therapies are an order of magnitude more complex to develop, manufacture and distribute, if they are to remain safe and effective.

3. Even for the more straight-forward non-Advanced Therapy products, it takes 1 – 1.5 years for FDA to evaluate and approve an application to market a new drug. The EUAs were approved within weeks.

4. On average, it takes 10 – 12 years to develop a new drug once discovery research has identified a development candidate. The SARS-CoV-2 injections were developed and manufactured in unit-dose quantities in the billions, within 6 – 9 months.

5. Please refer to US GAO Report GAO-07-49, titled *NEW DRUG DEVELOPMENT: Science, Business, Regulatory, and Intellectual Property Issues Cited as Hampering Drug Development Efforts* for further details on typical timelines.[iii]

6. The SARS-CoV-2 injections accelerated timescales could not possibly have been achieved without dangerous shortcuts being taken in the licensing process and

operation of the manufacturing and distribution supply chains post-approval. There can be no doubt that errors, omissions, adulteration of materials and products, would have been rife, leading to misbranding and patient harm.

7. This is because the stages involved in developing the manufacturing process for a new drug must be carried out in series (a predefined sequence from preclinical testing to final approval). To explain, initially preclinical safety studies on the manufactured drug substance must be carried out in animal models. These involve small quantities and a limited supply chain structure for the drug substance only. If the drug substance is proven safe, trials can begin in humans.

8. Larger quantities and batches are typically required to cover phase 1 and phase 2 requirements. As production is scaled up, or the process may be adjusted for technical or other reasons, the manufactured product can change in molecular structure. It is quite possible that a product that is safe at small scale, can turn toxic, or less potent, at a larger scale. New safety studies in animal models must be carried out on the new process. Again, for phase 3 studies, as the number of patients on studies increases significantly, larger quantities and batches are required. There may also be another scale up prior to approval and launch to provide the much great quantities for commercial supply. The public has repeatedly been told that the trials for the Sars-Cov-2 injections took place in parallel instead of in series, to speed up the process. It is my opinion that by developing the injections in this way, without following the accepted protocols, by proceeding through the steps in order, the vital checks on scaling up the

injections will have been missed. It is possible that the finished Product will be toxic to the recipient, or inactive. In my opinion, this is dangerous and leads to serious safety concerns.

9. The rolling review conducted all three phases of clinical trials in parallel and precluded any testing of manufactured drug substance safety prior to administration in humans. In my professional opinion this amounts to gross negligence by those involved. FDAs long established licensing process includes Module 3, the chemistry, manufacturing, and controls (CMC) section of the electronic Common Technical Document (eCTD). The eCTD is the electronic document that must be submitted by applicants to market a new drug, as laid down in CFR Title 21.[iv] It is unprecedented for FDA to forgo this evaluation, as it is there to ensure patients are kept safe from harm.

10. The evaluation of Module 3 requires FDA to scrutinize a massive amount of data related to the supply chain, as submitted by the sponsor company in the eCTD (BioNTech and Moderna). Those data include, but are not limited to, details of in-house manufacturing, third-party organizations such as contract development and manufacturing organizations (CDMOs) and contract research organizations (CROs), suppliers of materials and products along with their specifications, process development protocols, process validation reports, analytical methods development protocols, and a host of other data and information related to activities that fall within the CGMP umbrella, and other industry recognized standards, such a USP <1079>, Good Storage and Distribution Practices for Drug

Products.

11. The SARS-CoV-2 injections also required a greater degree of scrutiny as they are biological products, not the simpler small molecule products produced using industrial chemistry. Biological products are manufactured from living things, which means they are inherently unstable and subject to significant variation in clinical performance depending on the facility and equipment (such as transfer tubing and stainless-steel vessels) they are manufactured in. Their clinical performance also varies with the potency (titer) of input materials and changes to environmental temperature outside a safe range (such as -80°C to -60°C for the BioNTech injections). In summary, biological products can become toxic or ineffective during manufacture throughout the supply chain, and also cannot be considered clinically 'equivalent' to other apparently similar biologic products without bioequivalence testing. The accelerated development timelines would not have allowed that to take place, posing another huge risk to safety if they are used interchangeably.

12. The relevant FDA licensing document that applies to the SARS-CoV-2 injections is titled Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs). This applies to the companies wishing to obtain an approval of an investigational new drug (IND) application to study their drug in humans, referred to here.[v]

13. Section V, titled: MANUFACTURING PROCESS AND CONTROL INFORMATION (MODULE 3 OF THE CTD), details the data and information required to be

evaluated by FDA prior to any studies in humans. The workload is substantial and would certainly require at least the 3 years typical timeline in 1. above. For such a complex biological product classed as an advanced therapy, it would be reasonable to expect it to take considerably longer.

14. Based on the above and to ensure at least a minimum level of scrutiny, FDA should have carried out pre-approval inspections (PAIs) on ALL drug substance and drug product manufacturers, whether they be owned by the sponsor companies or working under contract to the sponsor companies. This has always been a mandatory component of FDAs drug approval process, as these steps are pivotal to the safety and efficacy of a drug. If this had been done, it is certain that the FDA inspectors, who are invariably highly experienced at getting to the core of CGMP issues, would have identified a host of critical observations, calling for immediate cessation of activities.

15. From my industry knowledge and experience, the Pfizer/BioNTech drug substance was manufactured at Wyeth Biopharma, Andover, US; BioNTech Manufacturing, GmBH Germany; and Rentschler BioPharma SE, Laupheim, Germany. For the drug product, it was Pfizer Manufacturing Belgium NV, Puurs, Belgium.

16. In the case of Moderna, Lonza, Visp, Switzerland manufactured the drug substance [vi] and Catalent Pharma Solutions, Bloomington, US manufactured the drug product.[vii]

17. There have been FDA inspections post-approval, and these have been deeply concerning, raising a red flag over BioNTech's and Moderna's capability to provide

the required oversight of the entire supply chain. The findings of two post-EUA FDA inspections of Rentschler BioPharma SE, Laupheim, Germany (BioNTech drug substance) and Catalent Pharma Solutions, Bloomington, US (Moderna drug product), published in the industry press, were alarming, to say the least: *Rentschler slapped with FDA Form 483 citing lax manufacturing procedures*[viii] and *Moderna's new booster launch tripped up by production issues at Catalent plant.*[ix] There is also further evidence of Catalent's CGMP violations, suggesting these are not isolated incidents: *Catalent's Belgium operations get a second FDA scolding within 1-year span*[x]

18. For convenience, the FDA inspection reports quoted in the articles, known as FDA Form 483s, are referenced separately here. [xi] [xii] [xiii]

19. There is yet more evidence of contract development and manufacturing organizations (CDMOs) engaged in manufacture of SARS-CoV-2 injections failing foul of FDA regulations. In this article*, Emergent's Covid vaccine problems more extensive than previously known,*[xiv] an example of many publishers covering the story, Emergent mixes together two different products being manufactured on behalf of two different companies. This again raises a red flag in respect of the sponsors of the SARS-CoV-2 injections being capable of proper oversight of its contractors.

20. The FDA Form 483s lead me to conclude that ALL the organizations inspected had deep-rooted systemic issues that would take years to remediate. It was the responsibility of BioNTech and Moderna to take immediate action, as clinical trial

sponsors. In practice, they did nothing. I can only conclude that both companies would have been incapable of taking appropriate action anyway, even if it was prepared to do so. This is because they do not have in their employ the skills and experience required to manage fully outsourced (virtual) biological product supply chains.

21. I have referred to the history of BioNTech published on its website, noting the claimed timelines:

    i. 2008 - BioNTech is founded by Prof. Ugur Sahin, M.D., Prof. Özlem Türeci, M.D., and Prof. Christoph Huber, M.D., to develop and produce treatments for individualized cancer immunotherapy. (Seed round 180m USD)

    ii. 2012 - Start of the first Phase 1 clinical trial with RNA immunotherapy in melanoma, today known as our FixVac approach

    iii. 2013 - Start of the first Phase 1 clinical trial with RNA Immunotherapy in melanoma – the first trial of an individualized immunotherapy (iNeST) in humans.

    iv. 2014-2018 - Strategic collaborations across the pipeline (Bayer Animal Health, Genentech, a member of the Roche Group, Genmab, Siemens, Sanofi, Regeneron, Genevant, Pfizer, University of Pennsylvania).

    v. 2018 - Completion of 270 million USD Series A financing round.

    vi. 2019 - BioNTech becomes a publicly traded company on the NASDAQ Global Select Market under the ticker symbol BNTX.

    vii. 2020 - Beginning of “Project Lightspeed” to quickly develop a safe and

effective vaccine to address the emerging SARS-CoV-2 pandemic. The Pfizer-BioNTech COVID-19 vaccine is the first vaccine to receive emergency use authorization following a worldwide Phase 3 trial.

viii. 2020 - Publication of first peer-reviewed paper for the COVID-19 vaccine candidate BNT162b1

ix. 2020 - Establishment of BioNTech Manufacturing Marburg to produce COVID-19 vaccine, which became one of the largest mRNA manufacturing facilities in 2021.

22. The above timeline shows that in the first five years, BioNTech had only completed one safety study in a cancer indication (skin), nothing for an infectious disease, such as a coronavirus. A phase 1 study provides no evidence of efficacy. Why then would all the companies listed above strike deals with BioNTech between 2014 – 2018? My prima facie conclusion is that they were acting in collusion with the larger pharmaceutical companies in 16 iv. above to provide support. Further investigations would be required to confirm this.

23. The same applies to Moderna. Incorporated in 2010, Moderna signed a lease to build a 200,000 sq ft GMP mRNA clinical manufacturing facility in Norwood, MA., in 2016. They were, however, still at the discovery research phase with their mRNA technology and in no position to embark on trials in humans—yet they did.

24. This appears hugely problematic, and normally the premises of small companies developing drugs would be inspected by FDA to cover all areas where they impact CGMP and other quality standards, such as Good Clinical Practice (GCP) and

Pharmacovigilance procedures. If this has not happened, then it should take place urgently.

25. Moving from manufacture to product distribution, there is significant evidence of serious violations in the storage and transportation of the temperature sensitive biological materials and products at each stage in the supply chain. The main stages are:

    i. Manufacture of starting materials (animal cells)
    ii. Upstream processing
    iii. Downstream processing
    iv. Packaging and labelling
    v. Distribution through wholesaler networks
    vi. Patient administration by qualified staff

26. When transporting materials and products from one stage to the next, the temperature range registered with FDA must be always adhered to during the journey. Temperature monitors must be positioned inside the packaging, to provide a real-time trace of the temperatures. Monitors must be activated at the start of the journey and deactivated at the end, where the trace is downloaded for inspection. Any excursion(s) outside the range must be investigated to ensure quality has not been impacted. If quality is found to have been impacted, the material or product must be rejected and destroyed.

27. This is a long-running problem in the industry because the companies in the supply chain are different legal entities, so allocation of responsibilities for actioning the

requirements in 15. above pose a significant risk of breaking down. The STAT article offers an example of how this has occurred in practice. [xv] There can be no doubt that there have been numerous non-compliances here, especially given the accelerated timescale for development and manufacture.

28. Stages v. and vi. are arguably the most troubling of all. The industry routinely distributes drugs through a well-established wholesaler network that is dominated by the three companies, namely McKesson, AmerisourceBergen, and Cardinal Health. These companies have a long history of working within the highly regulated storage and distribution of drugs and are embedded into US Health systems, requiring staff to be trained and equipped to meet the standards required. The SARS-CoV-2 injections, because they were frozen down to temperatures below which the distribution network was geared up for, had to bypass this network. Instead, they were shipped directly, via third party logistics providers, to facilities such as hastily constructed vaccination centres, supermarkets, and other make-do facilities. Not only that, but the injections had not completed manufacture. They were sold into the distribution network by Pfizer and Moderna packaged in cardboard trays, in quantities of 195 vials per tray, with each vial containing multiple doses in need of saline diluent to be added. This involved untrained staff, with no quality system providing standard operating procedures to guide their work, to convert the part manufactured product into a single unit-dose for patient administration. It is impossible to overstate how dangerous this is. Never in the history of the industry have commercially available

licensed drugs been supplied in a form that cannot be administered to, or consumed by, a patient with significant finishing operations still to be carried out.

29. This means that the label claim normally awarded by FDA as the final stage of drug approval, could not have been determined for the EUAs, as there were further operations to be carried out in addition to those included in the eCTD. These additional operations, such as thawing of bulk vials, addition of saline diluent, mixing the product to a homogeneous consistency, will alter the performance of the injections. In my professional opinion, this renders all SARS-CoV-2 injections misbranded.

30. It is also appropriate to point out the primary responsibility for pharmacovigilance lay with the companies developing and selling the drugs. FDAs responsibility is to ensure these companies have an effective process in place to respond immediately to any report of a serious adverse event (SAE). Again, this can only be assessed by FDA physically inspecting each company's place of work, in this case BioNTech, Pfizer and Moderna.

31. I also have deep concern over conferences springing up hailing advanced (including gene) therapies as the future for the pharmaceutical industry. One such example is Advanced Therapies 2023, held in London March 14-15, with FDAs Peter Marks as a Keynote speaker. Next year's can be seen in the reference. [xvi]

32. This article from March this year is also troubling: Dr Marks FDA's Marks hopes to align global regulators to boost gene therapy.[xvii]

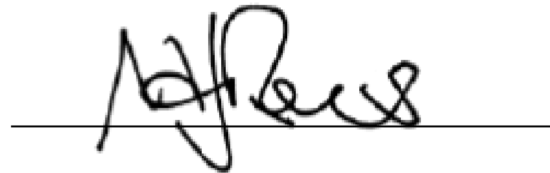
33. The companies involved in developing gene therapies appear to have little or no

understanding or experience of regulated drug development, supply chain management, and distribution of safe and effective drugs. In my professional opinion, FDA should be prioritising tackling the risks to patient safety that are eminently clear, following the catastrophic outcome with the experimental SARS-CoV-2 injections.

I am giving this declaration to: PROVIDE WRITTEN TESTIMONY TO SUPPORT LEGAL ACTIONS TO CONVENE A GRAND JURY AND TO PULL THE COVID-19 "VACCINES" UNDER CONSUMER PRODUCT PROTECTION STATUTES FOR LACK OF SAFETY AND EFFICACY. MISREPRESENTATION, MISBRANDING AND ADULTERATION/DEGRADATION, CAUSES.

I declare under penalty of perjury under the laws of the United Statesof America that the foregoing is true and correct.

Executed on this the 21st day of June 2023.



Hedley Rees, Director
PharmaFlow Limited

---

[i] Hedley Rees CV: https://www.dropbox.com/s/v3yks45fubbgbls/CV_HR_JULY_2022.pdf?dl=0
[ii] Overview Office of Tissues and Advanced Therapies and Division of Cellular and Gene Therapies Research Program, Raj K. Puri, M.D., Ph.D. Director, Division of Cellular and Gene Therapies Office of Tissues and Advanced Therapies https://www.fda.gov/media/140940/download
[iii] https://www.gao.gov/assets/gao-07-49.pdf
[iv] Electronic Common Technical Document (eCTD) v4.0 TECHNICAL CONFORMANCE GUIDE: https://www.fda.gov/media/135573/download Accessed February 23, 2023.
[v] Chemistry, Manufacturing, and Control (CMC) Information for Human Gene Therapy Investigational New Drug Applications (INDs): https://www.fda.gov/media/113760/download Accessed February, 23 2023.
[vi] Lonza and Moderna Enter New Agreement to Double Drug Substance Production for COVID-19

Vaccine in Visp: https://www.lonza.com/news/2021-04-29-05-00 Accessed February 23, 2023.
[vii] Moderna and Catalent Announce Long-Term Strategic Collaboration for Dedicated Vial Filling of Moderna's COVID-19 Vaccine and Clinical Portfolio: https://www.catalent.com/catalent-news/moderna-and-catalent-announce-long-term-strategic-collaboration-for-dedicated-vial-filling-of-modernas-covid-19-vaccine-and-clinical-portfolio/ Accessed February 23, 2023.
[viii] Rentschler slapped with FDA Form 483 citing lax manufacturing procedures: https://www.fiercepharma.com/manufacturing/rentschler-slapped-form-483-citing-lax-manufacturing-procedures Accessed February 20th 2023
[ix] Moderna's new booster launch tripped up by production issues at Catalent plant: https://www.fiercepharma.com/manufacturing/fda-cites-catalent-issues-indiana-plant-which-caused-delay-moderna-booster Accessed February 20th 2023
[x] Catalent's Belgium operations get a second FDA scolding within 1-year span: https://www.fiercepharma.com/manufacturing/catalents-belgium-operations-get-second-fda-scolding-inside-12-months Accessed February 20th 2023.
[xi] https://www.fda.gov/media/159164/download
[xii] https://www.fda.gov/media/161643/download
[xiii] https://www.fda.gov/media/162470/download
[xiv] Emergent's Covid vaccine problems more extensive than previously known. https://www.politico.com/news/2022/05/10/emergent-covid-vaccine-problems-00031266
[xv] Pfizer decision to turn off temperature sensors forced scramble to ensure Covid-19 vaccines kept ultra-cold: https://www.statnews.com/2020/12/17/pfizer-decision-to-turn-off-temperature-sensors-forced-scramble-to-ensure-covid19-vaccines-kept-cold/
[xvi] https://www.terrapinn.com/congress/advanced-therapies/index.stm
[xvii] https://www.reuters.com/business/healthcare-pharmaceuticals/fdas-marks-hopes-align-global-regulators-boost-gene-therapy-2023-03-29/

Attachment C

Publication by Kevin McKernan et al. 2023

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin
Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915

Several methods were deployed to assess the nucleic acid composition of four expired vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA contamination that exceeds the European Medicines Agency (EMA) 330ng/mg requirement and the FDAs 10ng/dose requirements. These data may impact the surveillance of vaccine mRNA in breast milk or plasma as RT-qPCR assays targeting the vaccine mRNA cannot discern DNA from RNA without RNase or DNase nuclease treatments. Likewise, studies evaluating the reverse transcriptase activity of LINE-1 and vaccine mRNA will need to account for the high levels of DNA contamination in the vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is still being investigated. Quantitative PCR assays used to track the DNA contamination are described.

Introduction

Several studies have made note of prolonged presence of vaccine mRNA in breast milk and plasma (Bansal et al. 2021; Hanna et al. 2022; Castruita et al. 2023). This could be the result of the stability of N1-methylpseudouridine (m1Ψ) in the mRNA of the vaccine. Nance *et al*. depict a vaccine mRNA synthesis method that utilizes a dsDNA plasmid that is first amplified in *E.coli* prior to an *in-vitro* T7 polymerase synthesis of vaccine mRNA (Nance and Meier 2021). Failure to remove this DNA could result in the injection of spike encoded nucleic acids more stable than the modified RNA. The EMA has stated limits at 330ng/mg of DNA to RNA (Josephson 2020-11-19). The FDA has issued guidance for under 10ng/dose in vaccines (Sheng-Fowler et al. 2009). Residual injected DNA can result in type I interferon responses and can increase the potential for DNA integration(Ulrich-Lewis et al. 2022).

Results

To assess the nucleic acid composition of the vaccines, vaccine DNA was deeply sequenced using two different methods. The first method used a commercially available New England Biolabs RNA-seq method that favored the sequencing of the RNA but still presented over 500X coverage for the unanticipated DNA vectors (Figure 1 and 2). The RNA-seq assemblies had truncated poly A tracts compared to the constructs described by Nance *et al*. The second method eliminated the RNA with RNase A treatment and sequenced only the DNA using a Watchmaker Genomics fragment library kit. The DNA focused assemblies delivered vector assemblies with more intact poly A tracts (Figure 3).

These assemblies were utilized to design multiplex qPCR and RT-qPCR assays that target the spike sequence present in both the vaccine mRNA and the DNA vector while also targeting the origin of replication sequence present only in the DNA vector (Figure 3). The assembly of Pfizer vial 1 contains a 72bp insertion not present in the assembly of Pfizer vial 2. This indel is known

for its enhancement to the SV40 promoter and its nuclear localization signal (Dean et al. 1999) (Moreau et al. 1981).

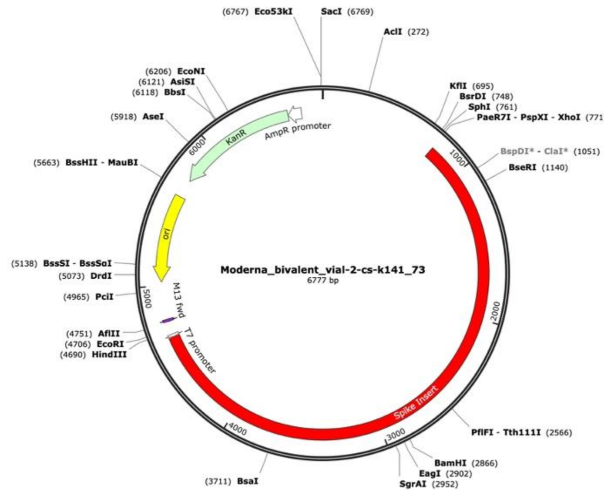


Figure 1. A Moderna vector assembly of an RNA-seq library with a spike insert (red), Kanamycin resistance gene (green) driven by an AmpR promoter and a high copy bacterial origin of replication (yellow).

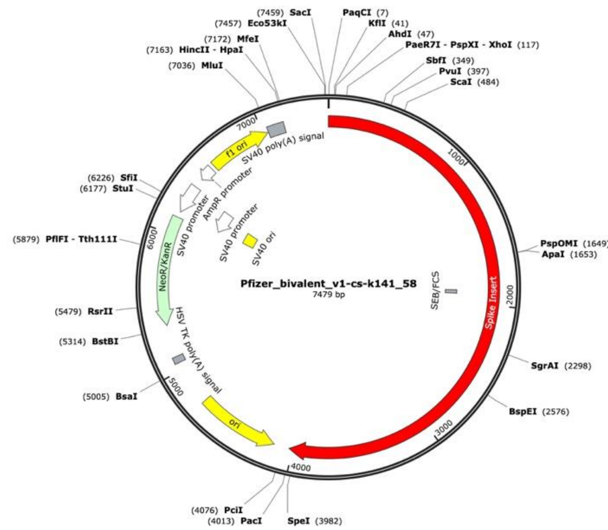


Figure 2. Pfizer bivalent vaccine assembly of the RNA-seq library. Annotated with SEB/FCS, spike insert (red), bacterial origin of replication (yellow), Neo/Kan resistance gene(green), F1 origin (yellow) and an SV40 promoter (yellow and white).

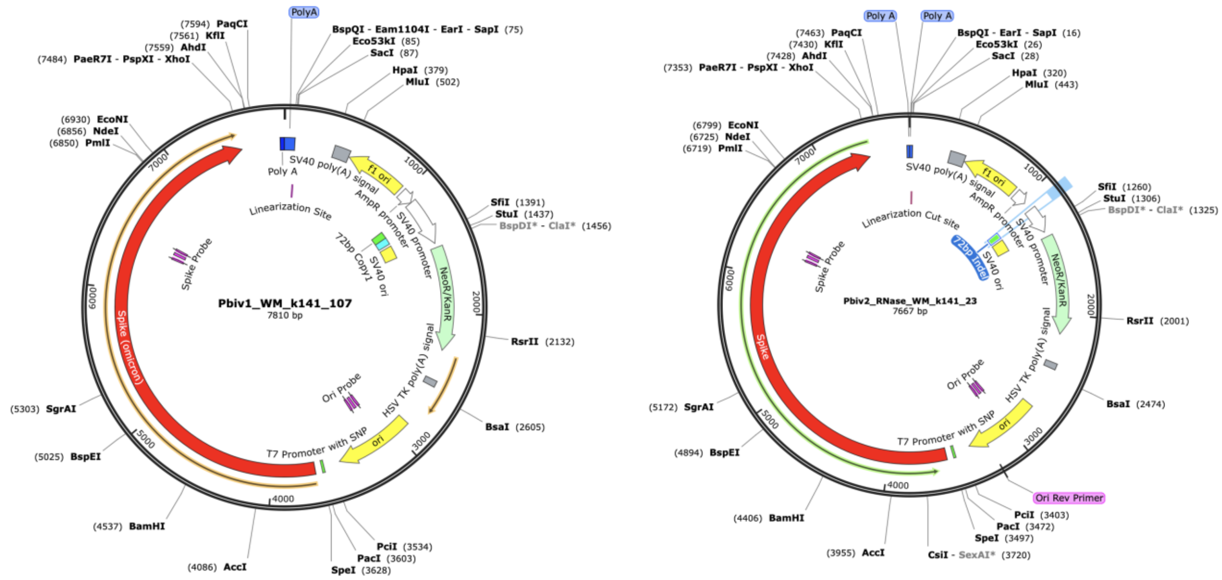


Figure 3. RNase treated vaccines were shotgun sequenced with Illumina (RNase-Seq not RNA-seq). Pfizer vectors from vial 1 (left) and vial 2 (right) contain a 72bp difference in the SV40 promoter (green and light blue annotation). qPCR assays are depicted in pink as Spike probe and Ori probe. The RNase sequencing provided better resolution over the Eam1104i linearization site and the Poly adenylation sequence. The vectors differ in the length of the polyA tail (likely sequencing artifact) and the 72bp indel.

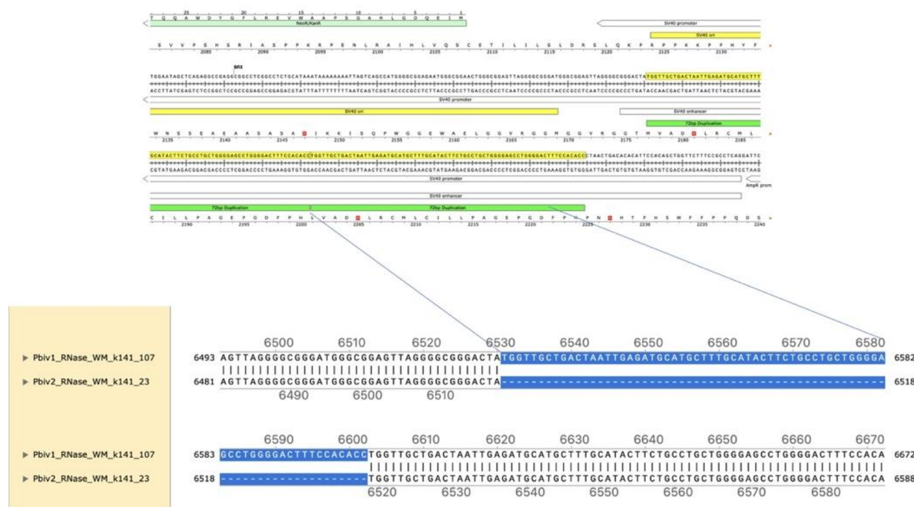


Figure 4. Local alignment of Pfizer vial 1 to Pfizer vial 2 vectors highlights the 72bp tandem duplication in blue.

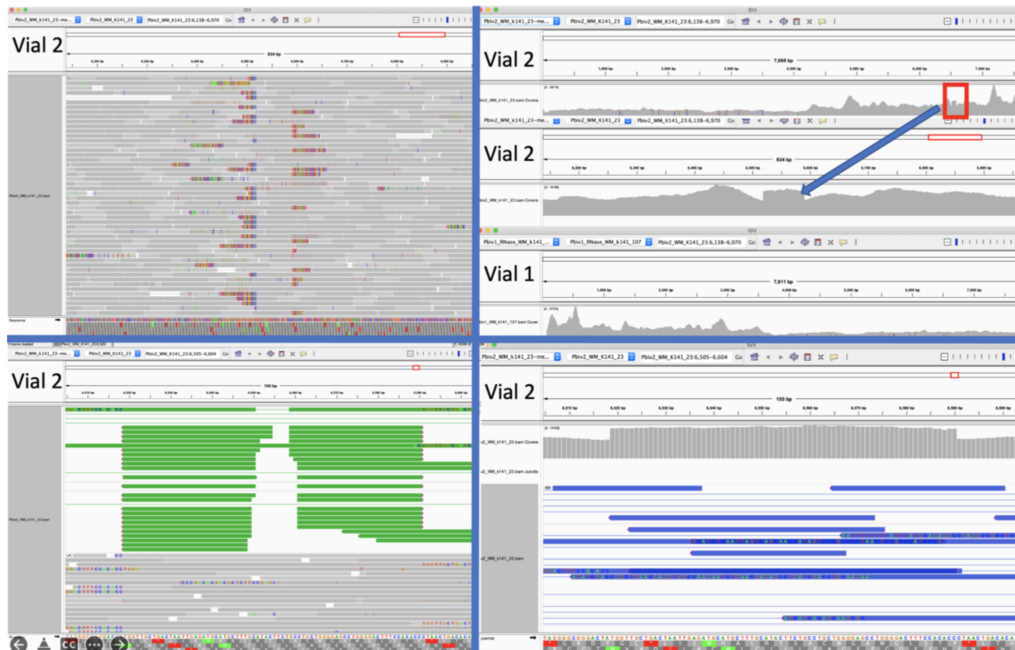


Figure 5A. Close inspection of the Integrative Genome Viewer (IGV) demonstrates the appearance of a 72bp insertion that is heteroplasmic in Pfizer vial 2. The upper left IGV view is a zoomed-out view where the colored marks depict the indel. The lower Left IGV view shows inverted paired reads as the 72bp insertion is a tandem repeat and paired reads shorter than 72bp can be mapped two different ways. Upper Right IGV view demonstrates a read coverage pile up or 'Plateau'. This occurs when the reference has one copy of the 72bp repeat and the sample has 2 copies. Note- In the upper right IGV depiction, the sequence in Vial 1 is in the opposite orientation in IGV as Vial 2. Lower right IGV view is a zoomed view of the upper right IGV screen.

Since the two Pfizer vials share the same lot number, finding a heterozygous copy number change between the two vials is unexpected. It was hypothesized that the appearance of a heteroplasmic copy number change is instead the result of the Megahit assembler collapsing what is actually two copies of the 72bp sequence into a single copy due to the insert sizes in the sequencing libraries being too short (105bp). It is noteworthy that the longer paired-end reads in the library resolve the 72bp tandem repeat.

When references have a single copy of the 72bp repeat and the sample has two copies of the repeat, reads should pile up to twice the coverage over the single copy 72bp loci as seen in Figure 5A. To test this hypothesis, we added a second 72bp sequence to the shorter plasmid assembly and observed that the reads map without artifact and no evidence of heteroplasmy (Figure 5B).

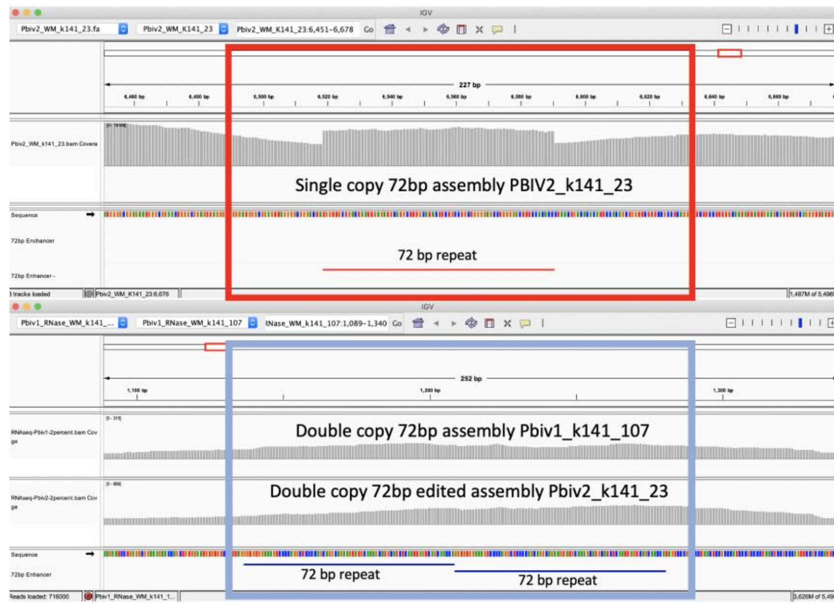


Figure 5B. IGV view of the read coverage over Pbiv2_k141_23 shows a discrete 72bp plateau in coverage (red rectangle). Editing the Pbiv2_k141_23 reference to include 2 copies of the 72bp sequence, and remapping the sequence data to this corrected sequence shows that the coverage over both vectors is more normal with no coverage plateau in Pfizer vial 2.

These data conclude that all Pfizer vectors contain a homoplastic 2 copy 72bp SV40 Enhancer associated with more robust expression and nuclear localization. The initial heteroplastic indel was an artifact of the Megahit assembler and short insert libraries.

To estimate the size of the DNA, the purified vaccines were evaluated on an Agilent Tape Station™ using DNA (genomic DNA screen tapes) and RNA based (high sensitivity RNA tapes) electrophoresis tapes.

Agilent Tape Station™ electrophoresis reveal 7.5 - 11.3 ng/µl of dsDNA compared to the 23.7 - 55.9ng/µl of mRNA detected in each 300µl sample. Qubit™ 3 fluorometry estimated 1-2.8ng/µl of DNA and 21.8ng - 52.8ng/µl of RNA. There is higher fragmentation seen in the DNA electrophoresis. The total RNA levels are less than the anticipated 30ug (100ng/µl) and 100ug (200ng/µl) doses suggesting a loss of yield in DNA and RNA isolation, manufacturing variance or RNA decay with expired lots.

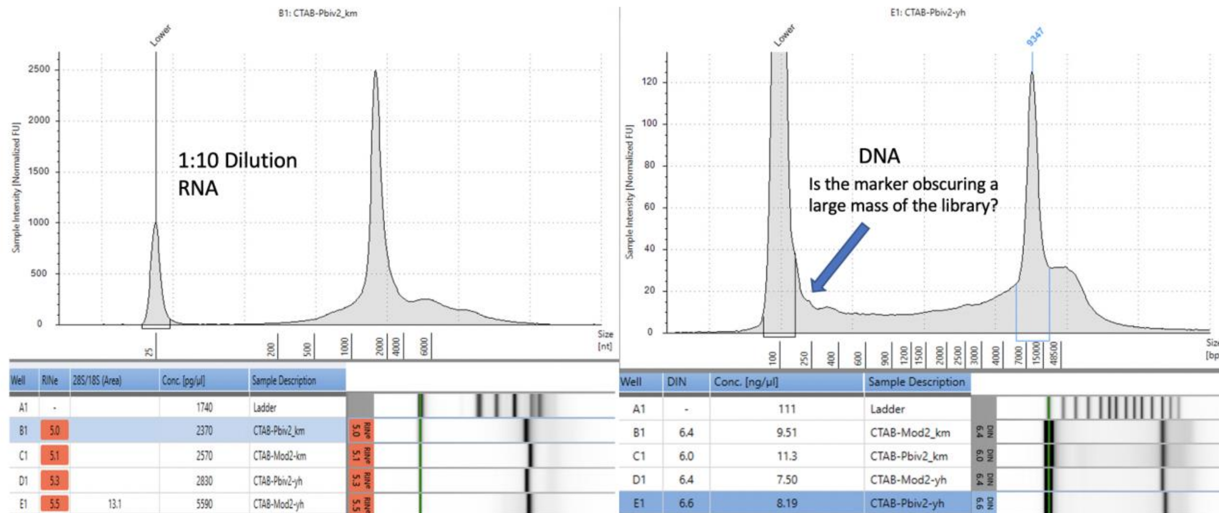


Figure 6. Agilent Tape Station™ electrophoresis demonstrates 23.7ng/µl – 55.9ng/µl of RNA (left). 7.5ng-11.3ng/µl are observed on DNA based Tape Station™. While the DNA electropherogram shows a peak suggestive of a full-length plasmid, this sample is known to have high amounts of N1-methylpseudouridine RNA present. DNA hybrids with N1-methylpseudouridine mRNA may provide enough intercalating dye cross talk to produce a peak. The sizing of the peak on the RNA tape on the left is shorter than expected. This may be the results of N1 methylpseudouridine changing the secondary structure or the mass to charge ratio of the DNA.

Quantitative PCR assays were designed using IDTs Primer Quest software targeting a region in the spike protein that was identical between Moderna and Pfizer spike sequences and a shared sequence in the vectors' origin of replication. This allowed the qPCR and RT-qPCR assessment of the vaccines. qPCR only amplifies DNA while RT-qPCR amplifies both DNA and RNA. Gradient qPCR was utilized to explore conditions where both targets would perform under the same cycling conditions for both RT-qPCR and PCR (gradient PCR data not shown).

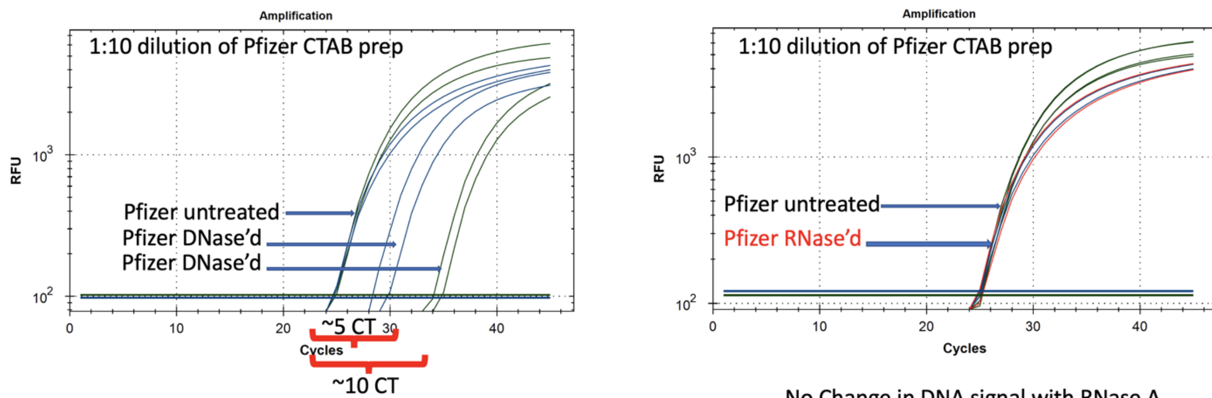


Figure 7. qPCR of Pfizer's bivalent vaccine with and without DNase I (left) and RNase A (right). Untreated mRNA demonstrates equal CTs for Spike and Vector assays as expected. Vector is more DNase I sensitive than the Spike suggesting the modRNA may inhibit nuclease activity of DNase I against complementary DNA targets. RNase A treatment doesn't alter the qPCR signal.

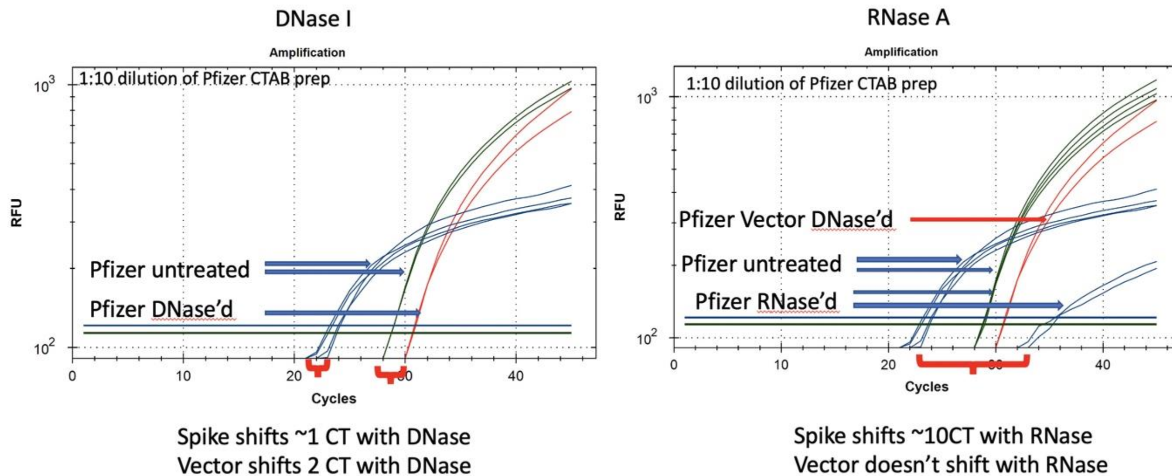


Figure 8. RT-qPCR amplifies both DNA and RNA. The untreated samples show a large CT offset with Pfizer Spike and Vector assays (Left Blue versus Green). This is anticipated as the T7 polymerization should create more mRNA over spike than over the vector. Small 1-2 CT shifts are seen with DNase I treatment. This is expected if the DNA is less than equal concentration of

nucleic acid in RT-PCR. RNase treatment (Right) shows a 10 CT offset but doesn't alter the DNA vector CT.

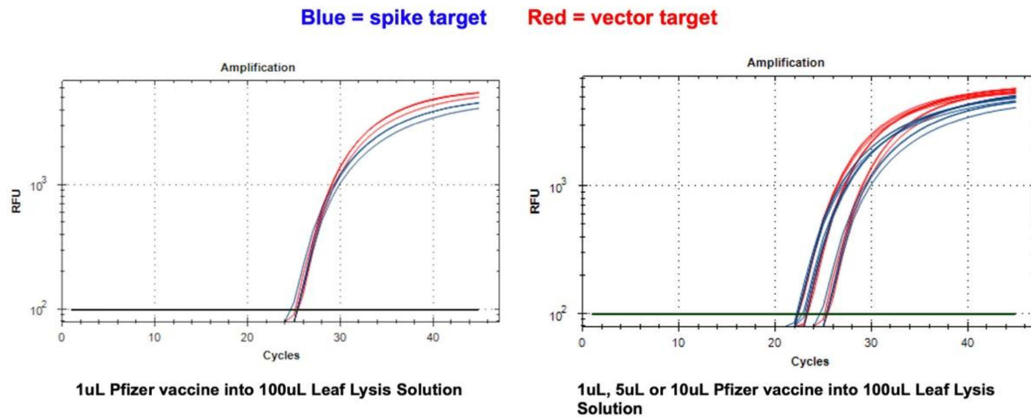


Figure 9. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 24 for both Vector and Spike targets in qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

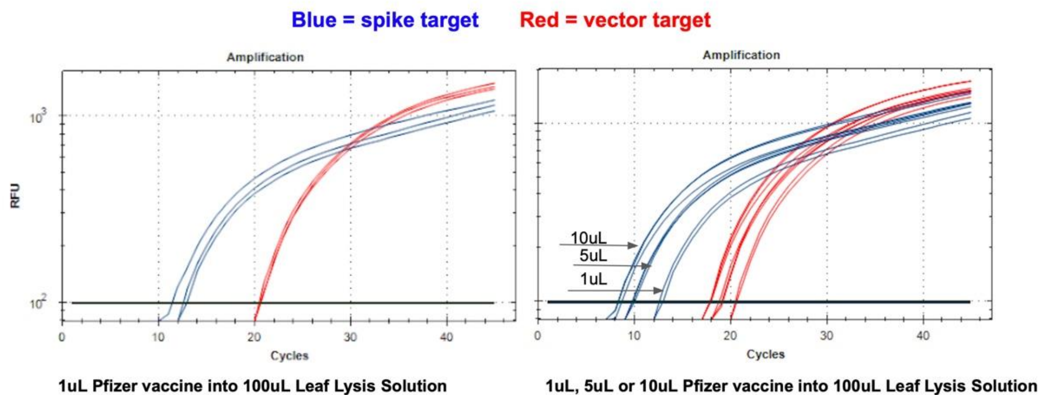


Figure 10. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 20 and 12 for both Vector and Spike targets in RT-qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

Moderna qPCR Results

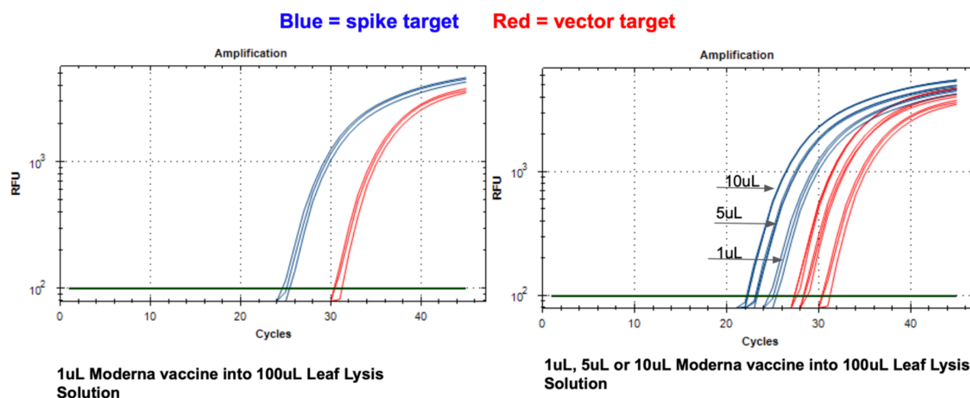


Figure 11. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with qPCR. This needs to be explored further as the assays provide equal CT scores on Pfizers' vaccines and the sequence of the amplicon is identical between the two vector origins. There are 2 mismatches in the spike amplicons between Moderna and Pfizer but none of the mismatches are under a primer or probe. The assay is responsive to 1,5,10µl of direct boil mRNA (Right).

Moderna RT-qPCR Results

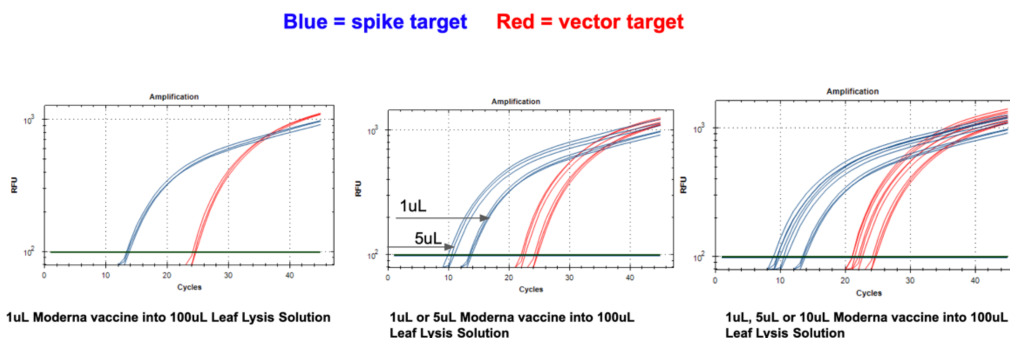


Figure 12. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with RT-qPCR. The large 10 CT shift between Spike and Vector needs to take into consideration that qPCR control shows a 5 CT offset. The boil preps can tolerate 1-10µl of vaccine (Middle and Right).

	Qubit DNA ng/µl	Qubit RNA ng/µl
Pbiv1	2.81	30.0
Pbiv2	1.47	52.8
Mod1	2.67	21.8
Mod2	1.04	49.0

Table 1. Qubit™ 3 Fluorometry estimates 1.04-2.8 ng/µl of dsDNA in the vaccines and 21.8ng-52.8ng/µl of RNA.

Synthetic templates were synthesized with IDT to build RT-qPCR standard curves to benchmark CTs to the mass of DNA in the reaction. This method uses ideal templates and fails to quantitate DNA molecules smaller than the amplicon size. As expected, this method delivers lower DNA concentration estimates than Qubit™ 3 fluorometry or Agilent Tape Station™. It also represents an ideal environment which doesn't capture the inhibition or primer depletion that can occur when large quantities of mRNA with identical sequence to your DNA target are co-present in a qPCR assay.

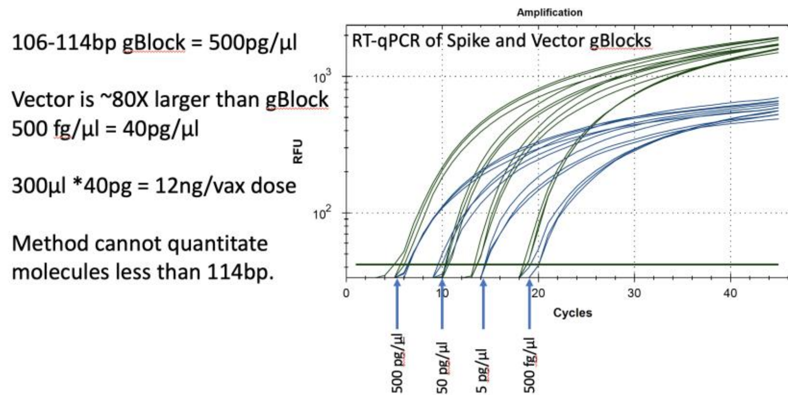


Figure 13. Two gBlocks were synthesized at IDT for Spike and Ori positive control templates used in an RT-qPCR assays. 10-fold serial dilutions were run in triplicate to correlate CT scores with picograms of DNA. The threshold is lowered from 10^2 for review of the background. CT of ~20 = 500fg/RT-qPCR reaction. Since 100bp targets only represent 1/80th of the vector DNA present as a potential contaminant, 500 fg/µl manifests in 40pg/µl of vector DNA. Any DNA that is DNase I treated and is smaller than the amplicon size cannot amplify or be quantitated with this method. This method will under quantitate DNase I treated samples compared to Qubit™ 3 or Agilent Tape Station™.

This work was further validated by testing 8 unopened Pfizer monovalent vaccines with both qPCR and RT-qPCR.

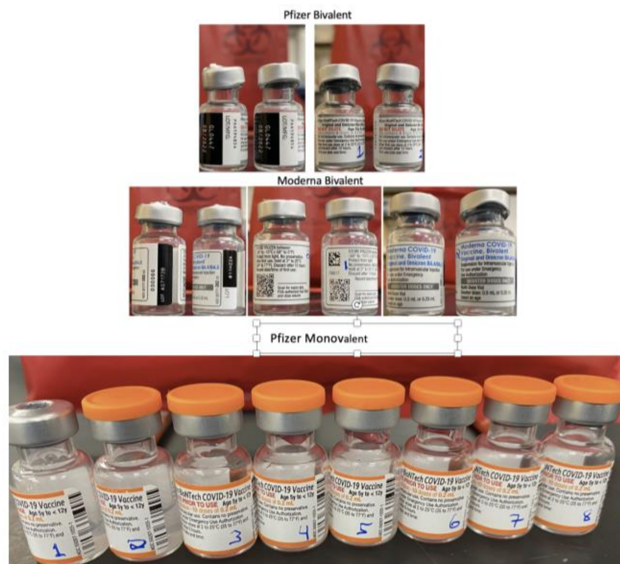


Figure 14. Moderna and Pfizer Bivalent vaccines (Top). 8 Monovalent Pfizer mRNA vaccines. These were unopened but past expiration (Bottom).

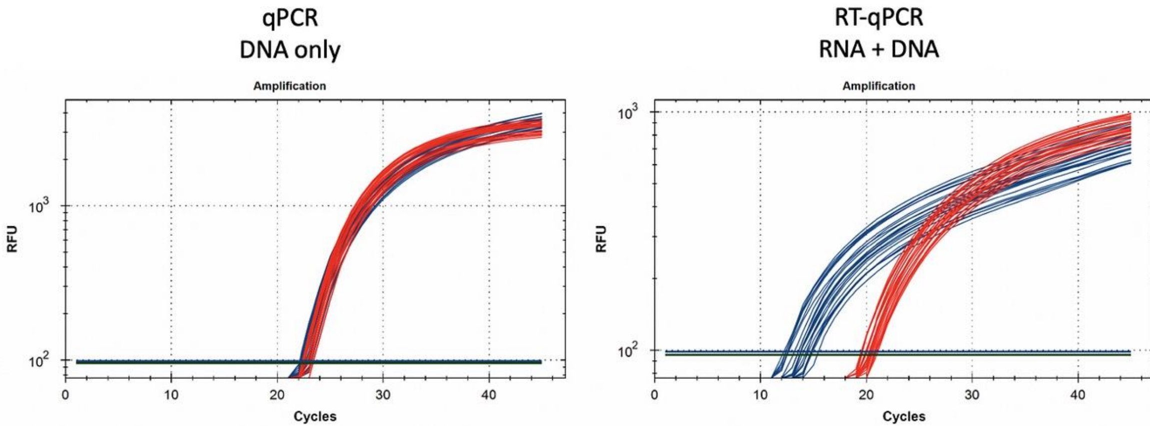


Figure 15. 1µl of vaccine boiled in 100µl of Leaf Lysis buffer was subjected to qPCR (left) and RT-qPCR (right) for Vector (red) and Spike (blue). 8 samples were tested in triplicate.

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 2. CT values for Spike and Vector during qPCR (DNA only). Standard deviation for the 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 and 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 3. CT values for Spike and Vector during RT-qPCR (RNA+DNA). Ratio of RNA:DNA ranges from 43:1 To 161:1. EMA allowable limit is 3030:1. This is 18-70 fold over the EMA limit.

Discussion

Multiple methods highlight high levels of DNA contamination in the both the monovalent and bivalent vaccines. While the Qubit™ 3 and Agilent Tape Station™ differ on their absolute quantification, both methods demonstrate it is orders of magnitude higher than the EMAs limit of 330ng DNA/ 1mg RNA. qPCR and RT-qPCR confirms the relative RNA to DNA ratio. An 11-12 CT offset should be seen between Spike and Vector RT-qPCR signals to represent a 1:3030

contamination limit (2^11.6 = 3100). Instead, we observe much smaller CT offsets (5-7 CTs) when looking at qPCR and RT-qPCR data with these vaccines. It should be noted that Qubit™ 3 and Agilent methods stain all DNA in solution while qPCR measures only amplifiable molecules without DNase I cut sites between the primers. The further apart you space the qPCR primers, the fewer Qubit™ 3 and Agilent detectable molecules will amplify. The primers used in this study are 106bp and 114bp apart, thus any molecules that are DNase I cut below this length will be undercounted with the qPCR methods relative to more general dsDNA measurements from Qubit™ 3 or Agilent Tape Station™.

This also implies that qPCR standard curves using 100% intact synthetic DNA standards will amplify more efficiently and thus undercount the total digested DNA contamination. For example, standard curves with 106-114bp synthetic templates provide CTs under 20 in the picogram range (not low nanogram range) suggesting large portions of the library are smaller than the minimum amplifiable size. Pure standards also do not contain high concentrations of modified mRNA with identical sequence which could serve as a competitive primer sink or inhibitor to qPCR methods.

Alternatively, the Qubit™ 3 and the Agilent Tape Station™ could be inflating the DNA quantification due to intercalating dye cross talk with N1-methylpseudouridine RNA. For this reason, we believe the ratio we observed when these molecules are more scrupulously interrogated with polymerases specific for each template type in qPCR and RT-qPCR is a more relevant metric. The EMA metric is also stated as such a ratio.

This also brings into focus if these EMA limits took into consideration the nature of the DNA contaminants. Replication competent DNA should arguably have a more stringent limit. DNA with mammalian promoters or antibiotic resistance genes may also be of more concern than just random background *E.coli* genomic DNA from a plasmid preparation (Sheng-Fowler et al. 2009). Background *E.coli* DNA was measured with qPCR and had CT over 35.

There has been a healthy debate about the capacity for SARs-CoV-2 to integrate into the human genome(Zhang et al. 2021). This work has inspired questions regarding the capacity for the mRNA vaccines to also genome integrate. Such an event would require LINE-1 driven reverse transcription of the mRNA into DNA as described by Alden *et al*. (Alden et al. 2022). dsDNA contamination of sequence encoding the spike protein wouldn't require LINE-1 for Reverse Transcription and the presence of an SV40 nuclear localization signal in Pfizer's vaccine vector would further increase the odds of integration. This work does not present evidence of genome integration but does underscore that LINE-1 activity is not required given the dsDNA levels in these vaccines. The nuclear localization of these vectors should also be verified.

Prior sequencing of the monovalent vaccines from Jeong *et al.* only published the consensus sequence (Dae-Eun Jeong 2021). The raw reads for this project are not available and should be scrutinized for the presence of vector sequence.

Given these vaccines exceed the EMA limits (330ng/mg DNA/RNA) with the Qubit™ 3 and Agilent data and these data also exceed the FDA limit (10ng/dose) with the more conservative qPCR standard curves, we should revisit the lipopolysaccharide (LPS) levels. Plasmid contamination from *E.coli* preps are often co-contaminated with LPS. Endotoxins contamination can lead to anaphylaxis upon injection (Zheng et al. 2021).

A limitation of this study is the unknown provenance of the vaccine vials under study. These vials were sent to us anonymously in the mail without cold packs. RNA is known to degrade faster than DNA and it is possible poor storage could result in faster degradation of RNA than DNA. RNA as a molecule is very stable but in the presence of metals and heat or background ubiquitous RNases, it can degrade very quickly. All of the vaccines in this study are past the expiration date listed on the vial suggesting more work is required to understand the DNA to RNA ratios in fresh lots. The publication of these qPCR primers may assist in surveying additional lots with more controlled supply chains. Studies evaluating vaccine longevity in breast milk or plasma may benefit from vector DNA surveillance as this sequence is unique to the vaccine and may persist longer than mRNA.

While the sequencing delivered full coverage of the plasmid backbones, it is customary to assemble plasmids from DNase I fragmented libraries. These methods have not discerned the ratio of linear versus circular DNA in the vials. While plasmid DNA is more competent and stable, linear DNA may have higher genome integration risks.

The intercalating dyes used in the Qubit™ 3 and Agilent systems are known to have low fluorescent cross talk with DNA and RNA but it is unknown to what degree N1-methylpseudouridine alters the specificity of these intercalating dyes. As a result, we have relied on the CT offsets between RT-qPCR and qPCR with the vector and spike sequence as the best relative assessment of the EMA ratio-metric regulation. These qPCR and RT-qPCR reagents may be useful in tracking these contaminants in vaccines, blood banks or patient tissues in the future.

Methods

Purifying the mRNA from the LNPs

LiDs/SPRI purification

100µl of each vial was sampled (1/3rd to 1/5th of a dose)

- 5µl of 2% LiDs was added to 100µl of Vaccine to dissolve LNPs
- 100µl of 100% Isopropanol
- 233µl of Ampure (Beckman Genomics)
- 25µl of 25mM $MgCl_2$ (New England Biolabs)

Samples were tip mixed 10X and incubated for 5 minutes for magnetic bead binding. Magnetic Beads were separated on a 96-well magnet plate for 10 minutes and washed twice with 200µl of 80% EtOH. The beads were left to air dry for 3 minutes and eluted in 100µl of ddH20. 2µl of eluted sample was run on an Agilent Tape Station™.

CTAB/Chloroform/SPRI purification of Vaccines

Some variability in qPCR performance was noted with our LiDs/SPRI purification method of the vaccines. This left some samples opaque and may represent residual LNPs in the purification. A CTAB/Chloroform/SPRI isolation was optimized to address this and used for further qPCR and Agilent electrophoresis. Briefly, 300µl of Vaccine was added to 500µl of CTAB (MGC solution A in SenSATIVAx MIP purification kit. #420004). The sample was then vortexed and heated for 5 minutes at 37°C. 800µl of chloroform was added, vortexed and spun at 19,000 rpms for 3 minutes. The top 250µl of aqueous phase was collected and added to 250µl of solution B and 1ml of magnetic binding buffer. Samples were vortexed and incubated for 5 minutes and magnetically separated. The supernatant was removed and the beads washed with 70% Ethanol two times. Samples were finally eluted in 300µl of MGC elution buffer.

Simple boil preparation for evaluating vaccine qPCR.

This boil prep process simply takes 1-10µl of the vaccine and dilutes it into a PCR compatible leaf lysis buffer and heats it (Medicinal Genomics part number 420208).

- 65°C for 6 minutes
- 95°C for 2 minutes

Library Construction for Sequencing

50µl of each 100µl sample was converted into RNA-Seq libraries for Illumina sequencing using the NEB NEBNext UltraII Directional RNA library Kit for Illumina (NEB#E7760S).

To enrich for longer insert libraries the fragmentation time was reduced from 15 minutes to 10 minutes and the First strand synthesis time was extended at 42°C to 50 minutes per the long insert recommendations in the protocol.

No Ribo depletion or PolyA enrichment was performed as to provide the most unbiased assessment of all fragments in the library. The library was amplified for 16 cycles according to the manufacturers protocol. A directional library construction method was used to evaluate the single stranded nature of the mRNA. This is an important quality metric in the EMA and TGA disclosure documents as dsRNA (>0.5%) can induce an innate immune response. dsRNA content is often estimated using an ELISA. Directional DNA sequencing offers a more comprehensive method for its estimation and was previously measured and 99.99% in Jeong et al. It is unclear how this may vary lot to lot or within the new manufacturing process for the newer bivalent vaccines.

RNase A treatment of the Vaccines

RNase A cleaves both uracils and cytosines. N1-methylpseudouridine is known to be RNAse-*L* resistant but RNase A will cleave cytosines which still exist in the mRNAs. This leaves predominantly DNA for sequencing. Vaccine mRNA that was previously sequenced and discussed here, was treated at 37°C for 30 minutes with 10µl of 20 Units/µl Monarch RNase A from NEB. The RNase reaction was purified using 1.5X of SenSATIVAx (Medicinal Genomics #420001). Sample were eluted in 20µl ddH20 after DNA purification. 15µl was used for DNA sequencing.

DNase treatment of the vaccines

50µl of CTAB purified vaccine was treated at 37°C for 30 minutes with 2µl DNase I and 6µl of DNase I buffer (Grim reefer MGC#420143). 2.5µl of LiDs Lysis buffer was added to stop the DNase reaction. Reactions were purified using 60µl 100% Isopropanol, 140µl Ampure, 15µl MgCl2. Magnetic beads were tip mixed 10 times, left for 5 minutes to incubate, magnetically separated and then washed twice with 80% EtOH.

Whole genome shotgun of RNase'd Vaccines.

15µl of the DNA was converted into sequence ready libraries using Watchmakers Genomics WGS library construction kit. This kit further fragments the DNA to smaller sizes making fragment length in the vaccines difficult to predict.

Qubit™ 3 Fluorometry

Qubit™ 3 fluorometry was performed using Biotum AccuBlue RNA Broad Range kit (#31073) and Biotum AccuGreen High Sensitivity dsDNA Quantitation Kit (#31066) according to the manufacturers instructions.

E.coli qPCR

Medicinal Genomics PathoSEEK™ E.coli Detection assay (#420102) was utilized according to the manufacturers instructions.

qPCR and RT-qPCR Spike Assay

- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Forward
- >AGATGGCCTACCGGTTCA
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Reverse
- >TCAGGCTGTCCTGGATCTT
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Probe
- >/56-FAM/CGAGAACCA/ZEN/GAAGCTGATCGCCAA/3IABkFQ/

qPCR and RT-qPCR Vector Origin Assay

- MedGen_Vax-vector_Ori_Forward
- >CTACATACCTCGCTCTGCTAATC
- MedGen_Vax-vector_Ori_Reverse
- GCGCCTTATCCGGTAACTATC
- MedGen_Vax-vector_Ori_Probe
- /5HEX/AAGACACGA/ZEN/CTTATCGCCACTGGC/3IABkFQ/

Elute primer to 100uM according to IDT instructions.

Make 50X primer-probe mix.

1. 25µl 100uM Forward Primer
2. 25µl 100uM Reverse Primer
3. 12.5µl 100uM Probe
4. 37.5µl nuclease free ddH20.

Use 15µl of this mixture in the **qPCR master mix** setup seen below. (0.5µl primer/probe per reaction)

Use 10µl of this mixture in the **RT-qPCR master mix** setup seen below.

Medicinal Genomics Master Mix kits used

1. https://store.medicinalgenomics.com/qPCR-Master-Kit-v3-200-rxns
2. https://store.medicinalgenomics.com/pathoseek-rt-qpcr-master-kit

Reaction setup for 30 reactions of qPCR

- 114µl Enzyme Mix (green tube)
- 24µl Reaction Buffer (blue tube)
- 246µl nuclease free ddH20
- 15µl of Primer-Probe set Spike
- 15µl of Primer-Probe set Ori

Use 13.8µl of above MasterMix and 5µl of purified sample (1µl Vax DNA/RNA + 4µl ddH20 if CT <15)

Reaction setup for 34 reactions of RT-qPCR

- 200µl Enzyme mix
- 96µl nuclease free ddH20
- 20µl RNase Inhibitor (purple tube)
- 4µl DTT (green tube)
- 10µl Primer-Probe set Spike
- 10µl Primer-Probe set Ori

10µl of MasterMix and 1µl of Vax DNA/RNA

Medicinal Genomics MIP DNA Purification Kit used

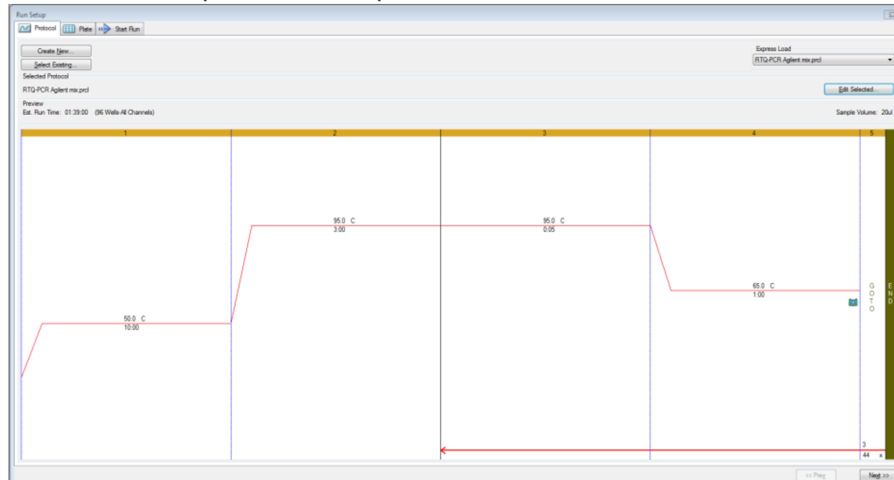
1. https://store.medicinalgenomics.com/SenSATIVAx-DNA-Extraction-Kit-200-reactions_2

he CTAB/Chloroform/SPRI based DNA/RNA isolation methods are described above.

Cycling conditions

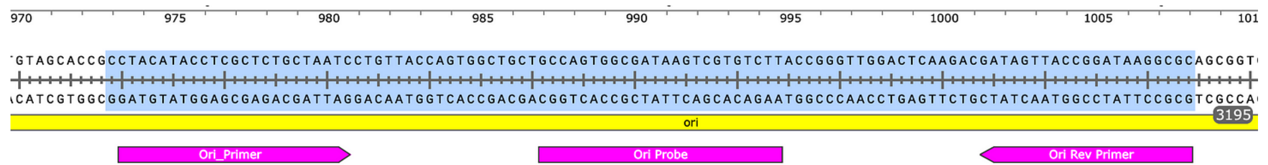
These conditions work for both qPCR and RT-qPCR. Note: The 50°C RT step can be skipped with qPCR. The MGC qPCR MasterMix kits used have a hot start enzyme which are unaffected by this 50°C step. For the sake of controlling RNA to DNA comparisons, we have put qPCR and RT-qPCR assays on the same plate and run the below program with the RT step included for all samples.

Cycling Conditions used for qPCR and RT-qPCR

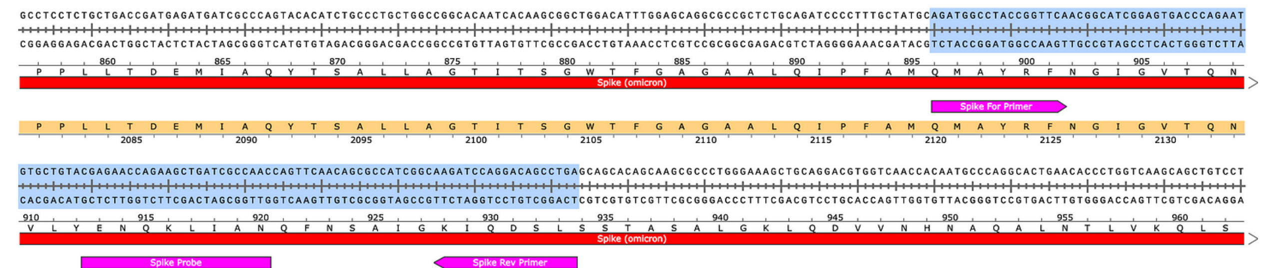


Sequences of amplicons for gBlock Positive Controls. Ori = 106bp, Spike = 114bp.

Ori target



Spike target



Sequencing Data

Raw Illumina Reads RNA-seq

- Pfizer Bivalent Vial 1 Forward reads
- Pfizer Bivalent Vial 1 Reverse reads
- Pfizer Bivalent Vial 2 Forward reads
- Pfizer Bivalent Vial 2 Reverse reads
- Moderna Vial 1 Forward reads
- Moderna Vial 1 Reverse reads
- Moderna Vial 2 Forward reads
- Moderna Vial 2 Reverse reads

Read files are run through sha256 (Hash and stash) and etched onto the DASH blockchain. The sha256 hash of the read file is spent into the OP_RETURN of an immutable ledger. If the hash of the file doesn't match the hash in these transactions, the file has been tampered with.

- Pfizer Vial 1 Forward hash
- Pfizer Vial 1 Reverse hash
- Pfizer Vial 2 Forward hash
- Pfizer Vial 2 Reverse hash
- Moderna Vial 1 Forward hash
- Moderna Vial 1 Reverse hash
- Moderna Vial 2 Forward hash
- Moderna Vial 2 Reverse hash

Megahit Assemblies

- Pfizer Vial 1
- Pfizer Vial 2
- Moderna Vial 1
- Moderna Vial 2

Illumina Reads mapped back to Megahit Assemblies

- Pfizer Vial 1 BAM File. Index File
- Pfizer Vial 2 BAM File. Index File
- Moderna Vial 1 BAM File. Index File
- Moderna Vial 2 BAM File. Index File

Q30 Filtered Illumina Reads (use these for transcriptional error rate estimates)

FastQ-Filter download: usage> fastq-filter -e 0.001 -o output.fastq input.fastq

- Pfizer bivalent Vial 1 Forward Reads
- Pfizer bivalent Vial 1 Reverse Reads
- Pfizer bivalent Vial 2 Forward Reads
- Pfizer bivalent Vial 2 Reverse Reads
- Moderna bivalent Vial 1 Forward Reads
- Moderna bivalent Vial 1 Reverse Reads
- Moderna bivalent Vial 2 Forward Reads
- Moderna bivalent Vial 2 Reverse Reads

Q30 BAM files. Q30 Reads mapped against Megahit assemblies

- Pfizer Vial 1 q30-BAM file. Index File
- Pfizer Vial 2 q30-BAM file. Index File
- Moderna Vial 1 q30-BAM file. Index File
- Moderna Vial 2 q30-BAM file. Index File

IGVtools error by base on q30 reads

Fields = Position in contig, Positive stand (+)A, +C, +G, +T, +N, +Deletion, +Insertion, Negative strand -A, -C, -G, -T, -N, -Deletion, -Insertion

- Moderna Vial 1

- Moderna Vial 2
- Pfizer Vial 1
- Pfizer Vial 2

Analysis pipeline

Reads were demultiplexed and processed with

- Trimgalore - Removes Illumina Sequencing adaptors.
- Megahit- assembles reads into contigs.
- Megahit for SARs-CoV-2
- Samtools- generates BAM files for viewing in IGV.
- Samtools stats used to calculate outie reads.
- BWA-mem- Short read mapper used to align reads back to the assembled references.
- SnapGene software- (www.snapgene.com)- Used to visualize and annotate expression vectors
- IGV- Integrated Genome Viewer used to visualize Illumina sequencing reads.

RNase Treated Libraries-BAM files

contig specific BAM files were created using samtools

```
samtools view -h input.bam contig_name -O BAM > contig.bam; samtools index contig.bam;
```

Samtools stats run on a each contig in each assembly.

```
for out_prefix in `ls *.sort.bam | perl -pe "s/.sort.bam//"`; do mkdir -p ${out_prefix}-samtools-stats; for contig in `samtools view -H ${out_prefix}.sort.bam  | grep "^@SQ"  | cut -f 2 | perl -pe "s/SN\://"`; do echo "Now calculating stats for ${contig}/$out_prefix..."; samtools stats ${out_prefix}.sort.bam $contig > ${out_prefix}-samtools-stats/${contig}-samtools-stats.txt; done; done
```

- Pbiv1_RNase_WM_k141_107.fa
- Pbiv1_RNase_WM_k141_107.bam
- Pbiv1_RNase_WM_k141_107.bam.bai
- Pbiv2_RNase_WM_k141_23.fa
- Pbiv2_RNase_WM_k141_23.bam

- Pbiv2_RNase_WM_k141_23.bam.bai

Author contributions
KJM- constructed the sequencing libraries, designed the qPCR assays, ran Qubit™ 3s and Agilent Tape Station™ and performed the analysis, drafted the manuscript.
YH-Optimized DNA isolations, Tape Station™ and qPCR results.
SM, LTK- assisted in demultiplexing and trimming the reads and assembly troubleshooting

Conflicts of interest- Authors of this paper are employees of Medicinal Genomics which manufacturers some of the qPCR and DNA isolation kits used in this study.

Acknowledgements.
We'd like to thank Lei Zhang for organizing the sequencing outsourcing. We'd like to thank Jessica Rose, Sabine Hazan, Jikkyleaks, Pathogenetics, Steve Massey, Valentine Bruttel, Lynn Fynn, Sasha Letypova, David Wiseman and Pharmacoconuts for helpful comments on this topic.

References

Alden M, Olofsson Falla F, Yang D, Barghouth M, Luan C, Rasmussen M, De Marinis Y. 2022. Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr Issues Mol Biol* **44**: 1115-1126.

Bansal S, Perincheri S, Fleming T, Poulson C, Tiffany B, Bremner RM, Mohanakumar T. 2021. Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines. *J Immunol* **207**: 2405-2410.

Castruita JAS, Schneider UV, Mollerup S, Leineweber TD, Weis N, Bukh J, Pedersen MS, Westh H. 2023. SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination. *APMIS* **131**: 128-132.

Dae-Eun Jeong MM, Karen Artiles, Orkan Ilbay, Andrew Fire*, Kari Nadeau, Helen Park, Brooke Betts, Scott Boyd, Ramona Hoh, and Massa Shoura*. 2021. Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273. *GitHub*.

Dean DA, Dean BS, Muller S, Smith LC. 1999. Sequence requirements for plasmid nuclear import. *Exp Cell Res* **253**: 713-722.

Hanna N, Heffes-Doon A, Lin X, Manzano De Mejia C, Botros B, Gurzenda E, Nayak A. 2022. Detection of Messenger RNA COVID-19 Vaccines in Human Breast Milk. *JAMA Pediatr* **176**: 1268-1270.

Josephson F. 2020-11-19. Rapporteur's Rolling Review assessment report. *Committee for Medicinal Products for Human Use* **EMEA/H/C/005735/RR**.

Moreau P, Hen R, Wasylyk B, Everett R, Gaub MP, Chambon P. 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. *Nucleic acids research* **9**: 6047-6068.

Nance KD, Meier JL. 2021. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent Sci* **7**: 748-756.

Sheng-Fowler L, Lewis AM, Jr., Peden K. 2009. Issues associated with residual cell-substrate DNA in viral vaccines. *Biologicals* **37**: 190-195.

Ulrich-Lewis JT, Draves KE, Roe K, O'Connor MA, Clark EA, Fuller DH. 2022. STING Is Required in Conventional Dendritic Cells for DNA Vaccine Induction of Type I T Helper Cell-Dependent Antibody Responses. *Front Immunol* **13**: 861710.

Zhang L, Richards A, Barrasa MI, Hughes SH, Young RA, Jaenisch R. 2021. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proceedings of the National Academy of Sciences of the United States of America* **118**.

Zheng Q, Wang T, Zhu X, Tian X, Zhong C, Chang G, Ran G, Xie Y, Zhao B, Zhu L et al. 2021. Low endotoxin E. coli strain-derived plasmids reduce rAAV vector-mediated immune responses both in vitro and in vivo. *Mol Ther Methods Clin Dev* **22**: 293-303.

Attachment A

Declaration of Pharma Specialist, Sasha Latypova

Excessive Variability in Pfizer's BNT162 Vaccine Formulation Batch-to-Batch

My Background and Experience:

I am a retired business executive with 20+ years of experience in pharmaceutical and medical device Research and Development (R&D) industry as well as in a broader data analytics field. Throughout my career my primary expertise was innovation in technologies used in drug development, as well as collection and analysis of data from global clinical trials. My experience covers all therapeutic areas of drug development. I was senior executive at several clinical research organizations (CROs) conducting data collection and analysis on behalf of pharmaceutical companies for the purpose of clinical trial data submissions to regulatory authorities such as FDA, EMA and other relevant government agencies. I have extensive experience working with the FDA staff on issues related to safety assessments of novel pharmaceuticals. Prior to working in the CRO field, I worked as analytical consultant in econometrics and litigation support, working primarily for pharmaceutical and medical device clients. I hold Master of Business Administration degree from Dartmouth College, Hanover, NH.

The following statements are based on my review of documentation that has been publicly disclosed from Pfizer, European Medicines Agency (EMA) and Food and Drug Administration (FDA) and relates to the Chemistry, Manufacturing and Controls (CMC) sections of Pfizer's BNT162 dossier. The documents were released due to a cyberattack on the EMA (see Attachment). The EMA acknowledged the release of the documents and did not dispute their authenticity. Furthermore, the British Medical Journal confirmed the contents of these documents with respect to the issues of integrity of the active ingredient discussed herein through correspondence with the EMA, MHRA, FDA, Health Canada and Pfizer.[1]

The rates of adverse events and deaths per manufacturing batch number are derived from CDC VAERS database.

My affidavit attests to the following facts identified in the documents, with evidence information provided below:

1. The modified RNA (mRNA) which is the active substance of Pfizer's vaccine BNT162b2 is allowed to vary in its integrity by up to 50% in the finished product.
2. Product impurities in the form of truncated mRNA, untranslated DNA and other unknown nucleic acid constructs have been allowed in the finished product in unspecified quantities.
3. As a result of the reckless widening of quality acceptance criteria for the integrity of active ingredient in manufacturing batches, there is a great variation in resulting formulations of final product as dispensed in vials. Furthermore, the contents of the vials are cut by hand into multiple doses by untrained and

[1] https://www.bmj.com/content/372/bmj.n627

unsupervised vaccinators who are working outside of the Good Manufacturing Practice compliance.

4. There is an excessive variation in the rates of adverse events and deaths observed post-vaccination for different manufacturing batches which far exceeds expected batch-to-batch variations for compendial pharmaceutical products, such as for example seasonal flu vaccines.

Evidence from EMA and Pfizer Documents:

Lack of mRNA integrity and product impurities (fragmented nucleic acid chains) were found in Pfizer's product days before it was authorized for market:

mRNA integrity, and conversely, its instability, is one of the most important variables relevant to all mRNA vaccines. Pfizer and BioNTech repeatedly stated that the efficacy of the product is highly dependent on the quantity of the sufficiently intact mRNA molecule. Even a minor degradation reaction, anywhere along a mRNA strand, can severely slow or stop proper translation performance of that strand and thus result in the incomplete expression of the target antigen.

Pfizer made several major changes to its manufacturing process going from small clinical scale manufacturing (Process 1) to commercial scale (Process 2) as described in the "Rapporteurs Rolling Review Report", p. 57 (full document in Attachment).

"Process 1

[…]two changes were made within Process 1 between nonclinical toxicology and Phase 1/2/3 process: the scale of the reaction and the site. The increase in scale was required to make sufficient material for clinical trials. The location changed from a non-GMP lab into GMP facilities. This process was based on BioNTech platform knowledge from other mRNA therapeutic programs.

Process 2

[…]The DNA template changed from a PCR template to linearized plasmid DNA in order to meet commercial demands. Additionally, the magnetic bead purification was replaced with proteinase K digestion and UFDF steps. The magnetic bead purification method was not scalable, but removed small molecule impurities (e.g. spermidine, DTT), residual DNA, and enzyme impurities (e.g. T7 polymerase, DNase I). […]"

These changes were performed without re-validation of the manufacturing process or re-running the preclinical and clinical studies to confirm comparability on safety and efficacy characteristics of the product. Importantly, these changes resulted in a substantial drop in the integrity of key active ingredient – mRNA molecule as measured by the %mRNA integrity and % of fragments (Late Migrating Species, LMC) in each manufactured batch. This was identified by the regulatory reviewers at EMA and FDA, and EMA specifically recorded this as a Major Objection #2, i.e. a regulatory flag that

required a resolution prior to the product approval. The discussions around this issue are recorded in numerous documents that were released from EMA, at the end of November 2020, including email exchanges between EMA staff and management (see Emails in Attachment). For example, a PowerPoint document from the meeting on November 26, 2020 between EMA and Pfizer/BioNTech describes the issue of mRNA integrity (see 20201126_BNT162b2_EMAmeeting14.pdf in Attachment).

In this meeting it was discussed that the batches manufactured with Process 2 had a much lower range of % intact mRNA and higher % of impurities – fragmented nucleic acid chains of various length and type (DNA and RNA). Specifically, p. 20 lists final product batches manufactured with both processes, ranging in mRNA integrity from 55% to 85% with the remaining % of volume occupied by uncharacterized fragments.

EMA regulatory concern with lack of mRNA integrity in Pfizer's product was evident. Specifically, on p. 4 the document states that:

"Significant differences between batches manufactured by DS Process 1 and 2 are observed for the CQA *[critical quality attribute]* mRNA integrity. In addition, the characterisation of BNT162b2 DS *[drug substance]* is currently not found acceptable in relation to this quality attribute. This is especially important considering that the current DS and DP *[drug product]* acceptance criteria allows (sic) for up to 50% fragmented species."

Further, on p. 5 the reviewers discussed the presence of uncharacterized fragmented nucleic chains, some long enough to translate into unknown proteins, and deemed them product impurities that required further characterization:

"Truncated and modified RNA species should be regarded as product-related impurities. Even though two methods, namely agarose gel electrophoresis and capillary gel electrophoresis (CGE), have been applied to determine RNA integrity of BNT162b2 DS *[drug substance]*, no characterisation (sic) data on truncated forms is presented. "

As a result of the manufacturing inconsistency, the clinical trial data collected using the Process 1 material was not deemed applicable to the material manufactured in Process 2. Several EMA reviewers wanted to understand the potential impact on safety and efficacy via bridging clinical studies (see Emails in Attachment). No such comparisons were done. Pfizer provided comparison of some chemical analyses from various batches, but no further characterization of the fragments of RNA and DNA or study of impact of these impurities on safety and efficacy of patients was provided.

EMA reviewers and Pfizer "resolved" this Major Objection by arbitrarily lowering the acceptance criteria for %mRNA integrity (see p.4):

"In addition, we are revising the RNA integrity specification for drug substance to >=60%, drug product release to >=55%, and drug product shelf life to >=50%. "

An extremely wide variation of the integrity of the active substance in bulk material (batch) of the product and abundant presence of uncharacterized impurities means that batches of different formulation - and thus different potency and safety profiles - are being produced. This variation is further amplified when the bulk material is filled in small quantities into vials. Each batch of Pfizer product contains approximately 300,000 vials filled with 0.45ml of drug product which may get varying quantities of intact and broken mRNA molecules. In addition, at the final step of administration, this variability is further exacerbated by dose preparation in a non-GMP environment by untrained and unsupervised staff at the vaccination centers.

Both the regulators and Pfizer to date have not disclosed the acceptable ranges for the key ingredients of the vaccine product, neither in bulk product nor in a vial (as dispensed), and claim “commercial secrets” that prevent them from doing so.

Evidence from adverse event reports (in VAERS database) analyzed by manufacturing lot number.

Manufacturing of pharmaceutical products is regulated by laws that are established to control within tight ranges acceptable criteria for the identity, quantity, quality, purity, potency and other characteristics of the product ingredients to ensure safety and conformity to the approved product labeling. It is expected that the product lot-to-lot, or batch-to-batch, is essentially the same. Therefore, when outcomes data such as rates of adverse events reported for each manufacturing lot is examined, it is expected that only minor variations from lot-to-lot may be observed. This is true for conventional pharmaceutical products and for traditional vaccines such as seasonal flu vaccines.

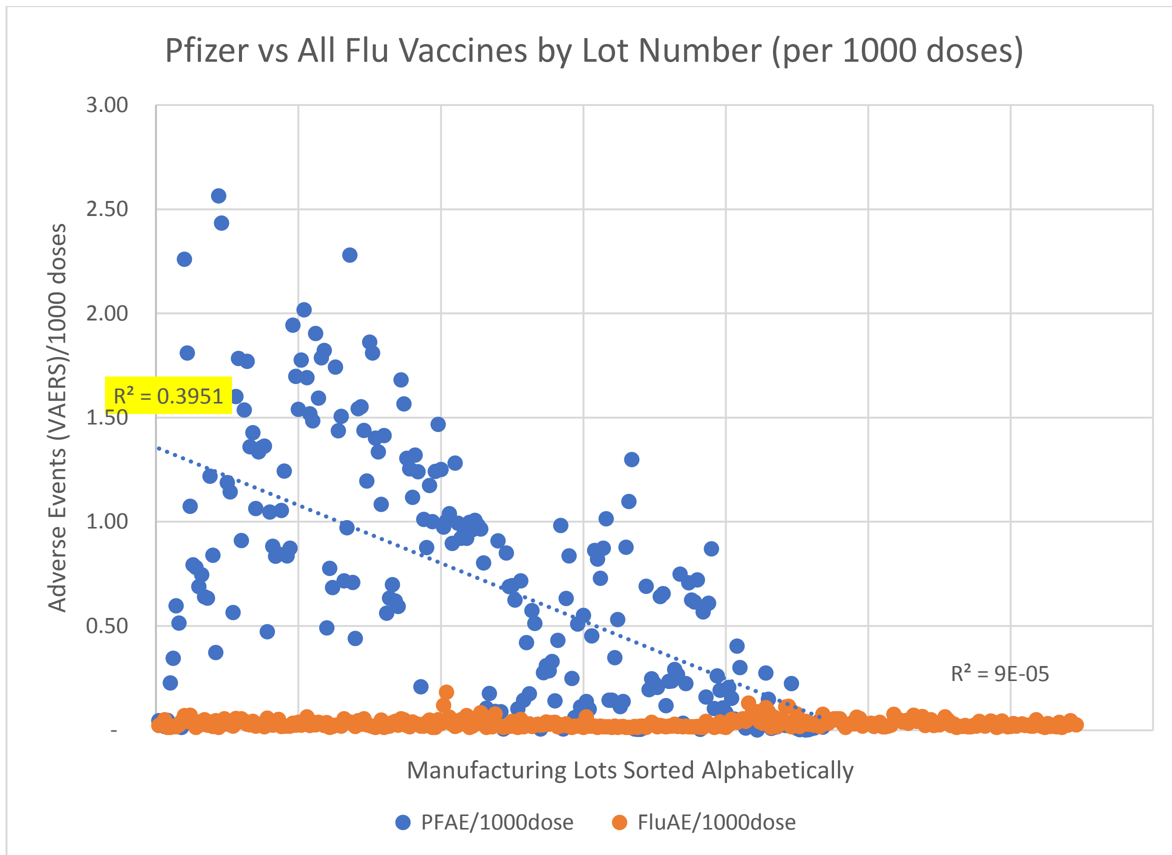
There is an excessive variation in the rates of adverse events and deaths observed post-vaccination for different manufacturing batches which far exceeds expected batch-to-batch variations for compendial pharmaceutical products, such as for example seasonal flu vaccines.

The graph below shows a comparison between the manufacturing lots of Pfizer’s BNT162b2 product and manufacturing lots of all seasonal flu vaccines released in 2019-2020. The lot numbers for Pfizer were verified with CDC and dates of manufacture and expiration were obtained. The flu vaccine lot numbers were obtained by downloading data from VAERS. Rates of adverse events reported for each lot are plotted against the lot number (not shown on X-axis for clarity), sorted alphabetically. Finally, the adverse event rates are expressed in “per 1000 doses” to normalize for the lot size.

As evident from this analysis, there is an excessive variability in the toxicity (rates of adverse events) for Pfizer product. The flu vaccine lots in comparison look very similar to each other and have overall a very low rate of adverse events. There is a large correlation between the adverse even rates for Pfizer lots with the lot number (R^2=0.4). This should not happen. There should be no difference in the safety (toxicity) of a

product depending on how its manufacturing lot is numbered. This does not exist for the flu vaccine lot numbers. Overall, the rate of adverse events per lot/dose adjusted is extremely high as can be visualized on the graph below.

The difference between the two sets of products is stark and cannot be explained by normal demographic variations such as age or underlying health status of the recipient. Flu vaccines are administered to approximately 50% of population, including to old and frail people with compromised health status as well.



In conclusion, the evidence presented in my statement shows that Pfizer's manufacturing quality acceptance criteria permit for an extremely large variation of the key ingredient (up to 50%) and allow for a substantial presence of uncharacterized impurities. This can be deemed as product adulteration with de-facto different formulations produced in different batches. This leads to overall large rates of toxicities, reported adverse events and to extreme variations of product safety (toxicity) parameters in different manufactured lots.

Alexandra (Sasha) Latypova

PO Box 2981

Stateline, NV, 89449, USA

latypova@hotmail.com

Attachment B

Declaration of Pharma Specialist Hedley Rees

DECLARATION: HEDLEY REES, B. ENG., HONS., EXECUTIVE MBA.

Pursuant to 28 U.S.C. § 1746, Hedley Rees, Bridgend, United Kingdom, hereby declares:

I am over the age of 18 and fully competent to make this declaration through my education, knowledge, experience, and training, of the facts stated in this declaration.

This declaration is submitted in support of: LEGAL ACTIONS TO CONVENE A GRAND JURY AND TO PULL THE COVID-19 "VACCINES" UNDER CONSUMER PRODUCT PROTECTION STATUTES FOR LACK OF SAFETY AND EFFICACY, MISREPRESENTATION, MISBRANDING, ADULTERATION AND DEGRADATION, CAUSES.

Based on my experience, knowledge, and training as a pharmaceutical and biologics supply chain management and regulatory specialist (CV here)[i], I explain below key aspects of the control of product and material contamination, and the potential impact of contamination on the identity, strength, quality, and purity of the SARS-CoV-2 injections:

1. Any company involved in the manufacture of prescription drugs (drugs) must adhere to CGMP regulations as defined in the US Code of Federal Regulations, Title 21.[ii]
2. The relevant sections applicable to drugs manufactured by chemical synthesis (small molecule) are *21 CFR PART 210 - CURRENT GOOD MANUFACTURING PRACTICE IN MANUFACTURING, PROCESSING, PACKING, OR HOLDING OF DRUGS; GENERAL*[iii] and *PART 211 - CURRENT GOOD MANUFACTURING PRACTICE FOR FINISHED PHARMACEUTICALS*[iv]
3. The SARS-CoV-2 injections are categorized as biological products, and in addition to 21 CFR PART 210/211, are also governed by 21 CFR PART 600 - BIOLOGICAL PRODUCTS: GENERAL[v] where biological products are defined as:

"Biological product means a virus, therapeutic serum, toxin, antitoxin, vaccine, blood, blood component or derivative, allergenic product, protein, or analogous product, or arsphenamine or derivative of arsphenamine (or any other trivalent organic arsenic compound), applicable to the prevention, treatment, or cure of a disease or condition of human beings."

4. Biological products are far more susceptible to quality issues than small molecule drugs. This is because they are made from living organisms such as animal and human cells, which can suddenly change character depending on the physical environment (eg temperature or humidity), methods of preparation and processing procedures.
5. Microbial, particulate and pyrogen contamination are ever present, critical risks in the manufacture of sterile injectables such as the SARS-coV-2 injections.
6. Adherence to PART 210/211/600 is essential to assure material and product quality.
7. § 211.113 *Control of microbiological contamination* states [vi]:

   "Appropriate written procedures, designed to prevent microbiological contamination of drug products purporting to be sterile, shall be established and followed. Such procedures shall include validation [proof they work as intended] of all aseptic and sterilization processes."
8. Contamination is classed as a quality deviation, and must recorded and justified, as per § 211.100 Written procedures; deviations.[vii]

   "(a) There shall be written procedures for production and process control designed to assure that the drug products have the identity, strength, quality, and purity they purport or are represented to possess. Such procedures shall include all requirements in this

subpart. These written procedures, including any changes, shall be drafted, reviewed, and approved by the appropriate organizational units and reviewed and approved by the quality control unit.

(b) Written production and process control procedures shall be followed in the execution of the various production and process control functions and shall be documented at the time of performance. Any deviation from the written procedures shall be recorded and justified."

9. The quality control unit, under § 211.22 "Responsibilities of quality control unit," [viii] must determine what measures are required. See below:

(a) There shall be a quality control unit that shall have the responsibility and authority to approve or reject all components, drug product containers, closures, in-process materials, packaging material, labeling, and drug products, and the authority to review production records to assure that no errors have occurred or, if errors have occurred, that they have been fully investigated. The quality control unit shall be responsible for approving or rejecting drug products manufactured, processed, packed, or held under contract by another company.

(b) Adequate laboratory facilities for the testing and approval (or rejection) of components, drug product containers, closures, packaging materials, in-process materials, and drug products shall be available to the quality control unit.

(c) The quality control unit shall have the responsibility for approving or rejecting all procedures or specifications impacting on the identity, strength, quality, and purity of the drug product.

(d) The responsibilities and procedures applicable to the quality control unit shall be in writing; such written procedures shall be followed.

9. The initial response must be to contain the outbreak. Production must be ceased immediately, and all production batches suspected of being contaminated must be placed in a quality status that prevent use, such as 'in bond' or 'quarantined'. The bonded production must be physically labelled clearly stating the inventory is not available for use. There should also be a review of batch manufacturing records (BMRs) of products that have already left the facility, to determine if there could be a need to alert downstream supply chain actors of any potential issues.

10. In parallel with containment activities, a deviation investigation must be instigated. The aim of the investigation is to establish the root cause of the contamination problem. This will involve much interchange of scientific, technical and supply chain information, review and approval by the appropriate organizational units, to be finally reviewed and approved by the quality control unit. Often, it takes weeks, or even months, to arrive at a decision on the root cause of a deviation.

11. Once root cause has been identified, a corrective and preventative action plan (CAPA) must be established and implemented via the organization's quality management system (QMS). This again can take weeks or months, even for the more straight forward small molecule drugs, as corrective standard operating procedures (SOPs) must be written, reviewed, and approved. Operators must then be trained in the new SOPs, sign that they have read and understood, and their training records updated.

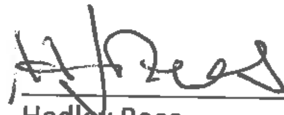
12. A contamination finding for a biological product is an order of magnitude more complex

FORM 483.

I am giving this declaration to: PROVIDE WRITTEN TESTIMONY TO SUPPORT LEGAL ACTIONS TO CONVENE A GRAND JURY AND TO PULL THE COVID-19 "VACCINES" UNDER CONSUMER PRODUCT PROTECTION STATUTES FOR LACK OF SAFETY AND EFFICACY. MISREPRESENTATION, MISBRANDING AND ADULTERATION/DEGRADATION, CAUSES.

I declare under penalty of perjury under the laws of the United Statesof America that the foregoing is true and correct.

Executed on this the 6 th day of April, 2023.



Hedley Rees

Director, PharmaFlow Limited

---

[i] https://www.dropbox.com/s/v3yks45fubbgbls/CV_HR_JULY_2022.pdf?dl=0

[ii] https://www.ecfr.gov/current/title-21

[iii] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-210

[iv] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211

[v] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-F/part-600

[vi] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211/subpart-F/section-211.113

[vii] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211/subpart-F/section-211.100

[viii] https://www.ecfr.gov/current/title-21/chapter-I/subchapter-C/part-211/subpart-B/section-211.22

[ix] https://www.fda.gov/vaccines-blood-biologics/report-problem-center-biologics-evaluation-research/biological-product-deviations

Attachment C

Publication by Kevin McKernan et al. 2023

Sequencing of bivalent Moderna and Pfizer mRNA vaccines reveals nanogram to microgram quantities of expression vector dsDNA per dose

Kevin McKernan, Yvonne Helbert, Liam T. Kane, Stephen McLaughlin
Medicinal Genomics, 100 Cummings Center, Suite 406-L, Beverly Mass, 01915

Several methods were deployed to assess the nucleic acid composition of four expired vials of the Moderna and Pfizer bivalent mRNA vaccines. Two vials from each vendor were evaluated with Illumina sequencing, qPCR, RT-qPCR, Qubit™ 3 fluorometry and Agilent Tape Station™ electrophoresis. Multiple assays support DNA contamination that exceeds the European Medicines Agency (EMA) 330ng/mg requirement and the FDAs 10ng/dose requirements. These data may impact the surveillance of vaccine mRNA in breast milk or plasma as RT-qPCR assays targeting the vaccine mRNA cannot discern DNA from RNA without RNase or DNase nuclease treatments. Likewise, studies evaluating the reverse transcriptase activity of LINE-1 and vaccine mRNA will need to account for the high levels of DNA contamination in the vaccines. The exact ratio of linear fragmented DNA versus intact circular plasmid DNA is still being investigated. Quantitative PCR assays used to track the DNA contamination are described.

Introduction

Several studies have made note of prolonged presence of vaccine mRNA in breast milk and plasma (Bansal et al. 2021; Hanna et al. 2022; Castruita et al. 2023). This could be the result of the stability of N1-methylpseudouridine (m1Ψ) in the mRNA of the vaccine. Nance *et al*. depict a vaccine mRNA synthesis method that utilizes a dsDNA plasmid that is first amplified in *E.coli* prior to an *in-vitro* T7 polymerase synthesis of vaccine mRNA (Nance and Meier 2021). Failure to remove this DNA could result in the injection of spike encoded nucleic acids more stable than the modified RNA. The EMA has stated limits at 330ng/mg of DNA to RNA (Josephson 2020-11-19). The FDA has issued guidance for under 10ng/dose in vaccines (Sheng-Fowler et al. 2009). Residual injected DNA can result in type I interferon responses and can increase the potential for DNA integration(Ulrich-Lewis et al. 2022).

Results

To assess the nucleic acid composition of the vaccines, vaccine DNA was deeply sequenced using two different methods. The first method used a commercially available New England Biolabs RNA-seq method that favored the sequencing of the RNA but still presented over 500X coverage for the unanticipated DNA vectors (Figure 1 and 2). The RNA-seq assemblies had truncated poly A tracts compared to the constructs described by Nance *et al*. The second method eliminated the RNA with RNase A treatment and sequenced only the DNA using a Watchmaker Genomics fragment library kit. The DNA focused assemblies delivered vector assemblies with more intact poly A tracts (Figure 3).

These assemblies were utilized to design multiplex qPCR and RT-qPCR assays that target the spike sequence present in both the vaccine mRNA and the DNA vector while also targeting the origin of replication sequence present only in the DNA vector (Figure 3). The assembly of Pfizer vial 1 contains a 72bp insertion not present in the assembly of Pfizer vial 2. This indel is known

for its enhancement to the SV40 promoter and its nuclear localization signal (Dean et al. 1999) (Moreau et al. 1981).

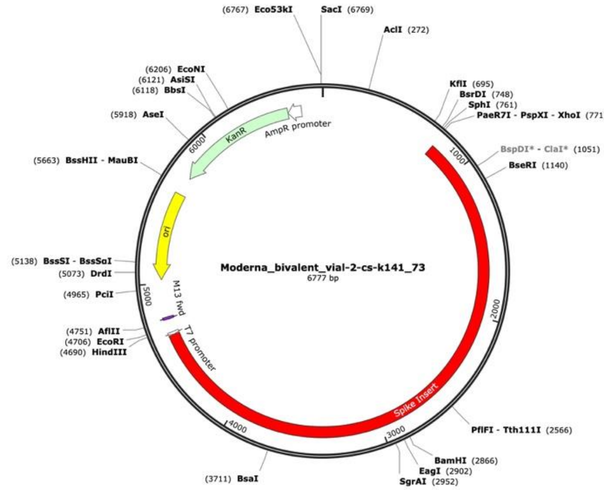


Figure 1. A Moderna vector assembly of an RNA-seq library with a spike insert (red), Kanamycin resistance gene (green) driven by an AmpR promoter and a high copy bacterial origin of replication (yellow).

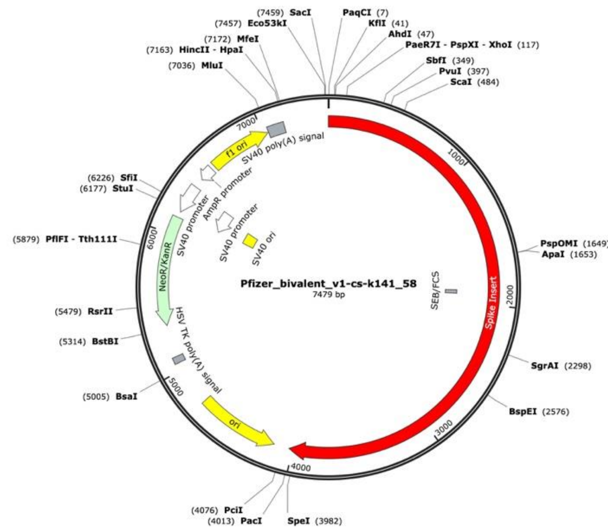


Figure 2. Pfizer bivalent vaccine assembly of the RNA-seq library. Annotated with SEB/FCS, spike insert (red), bacterial origin of replication (yellow), Neo/Kan resistance gene(green), F1 origin (yellow) and an SV40 promoter (yellow and white).

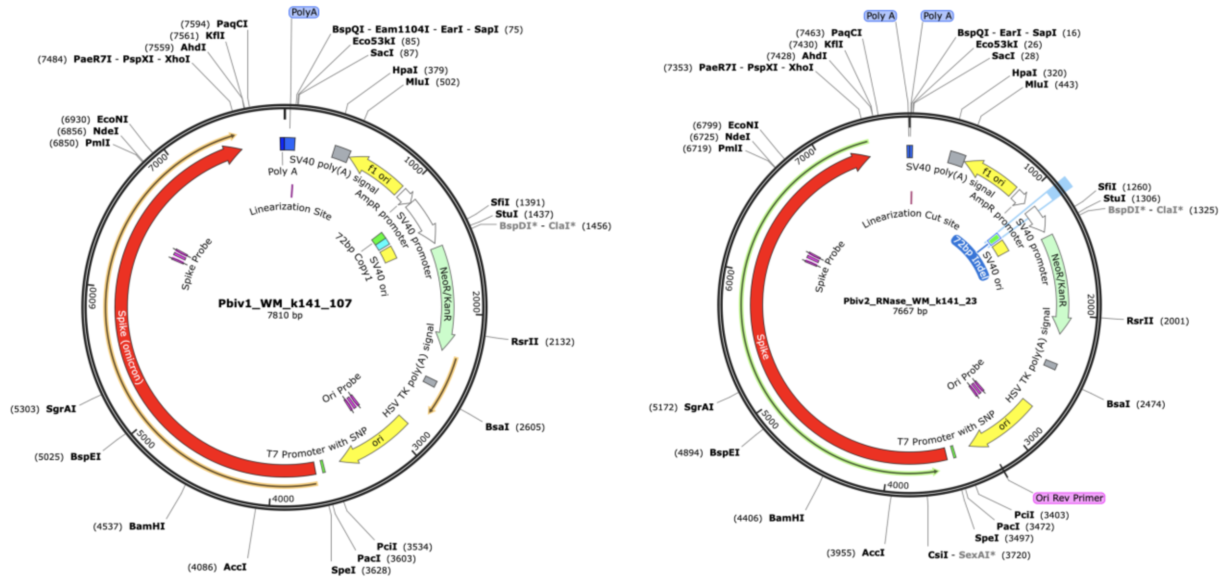


Figure 3. RNase treated vaccines were shotgun sequenced with Illumina (RNase-Seq not RNA-seq). Pfizer vectors from vial 1 (left) and vial 2 (right) contain a 72bp difference in the SV40 promoter (green and light blue annotation). qPCR assays are depicted in pink as Spike probe and Ori probe. The RNase sequencing provided better resolution over the Eam1104i linearization site and the Poly adenylation sequence. The vectors differ in the length of the polyA tail (likely sequencing artifact) and the 72bp indel.

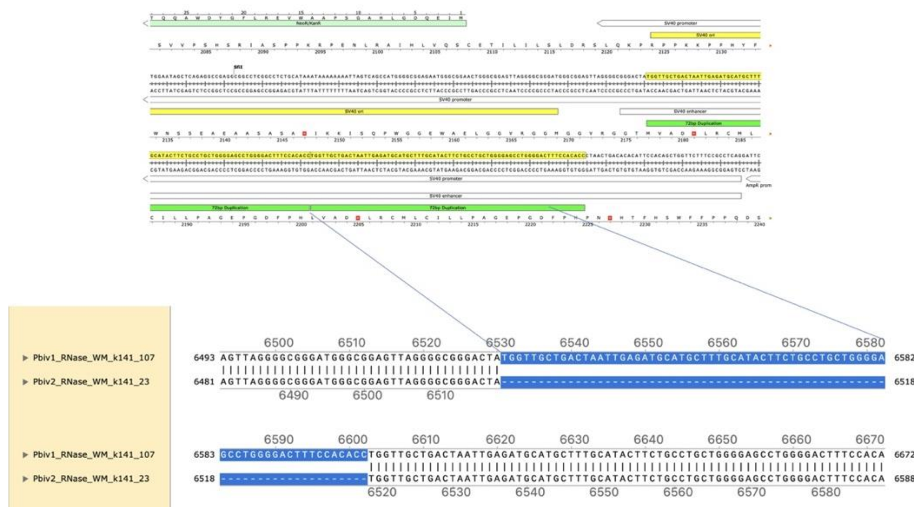


Figure 4. Local alignment of Pfizer vial 1 to Pfizer vial 2 vectors highlights the 72bp tandem duplication in blue.

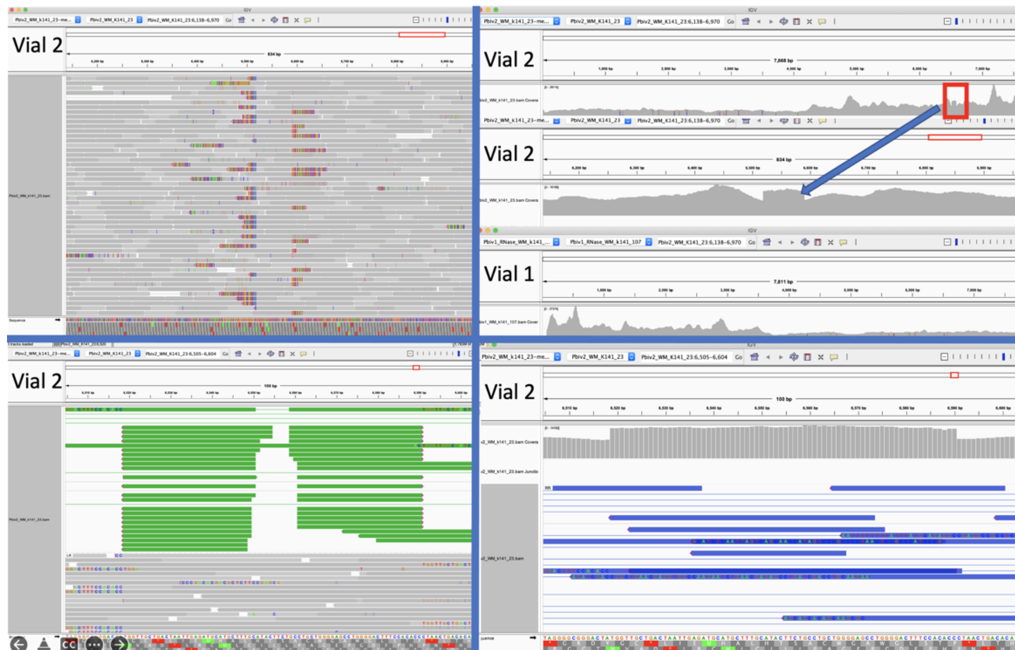


Figure 5A. Close inspection of the Integrative Genome Viewer (IGV) demonstrates the appearance of a 72bp insertion that is heteroplasmic in Pfizer vial 2. The upper left IGV view is a zoomed-out view where the colored marks depict the indel. The lower Left IGV view shows inverted paired reads as the 72bp insertion is a tandem repeat and paired reads shorter than 72bp can be mapped two different ways. Upper Right IGV view demonstrates a read coverage pile up or 'Plateau'. This occurs when the reference has one copy of the 72bp repeat and the sample has 2 copies. Note- In the upper right IGV depiction, the sequence in Vial 1 is in the opposite orientation in IGV as Vial 2. Lower right IGV view is a zoomed view of the upper right IGV screen.

Since the two Pfizer vials share the same lot number, finding a heterozygous copy number change between the two vials is unexpected. It was hypothesized that the appearance of a heteroplasmic copy number change is instead the result of the Megahit assembler collapsing what is actually two copies of the 72bp sequence into a single copy due to the insert sizes in the sequencing libraries being too short (105bp). It is noteworthy that the longer paired-end reads in the library resolve the 72bp tandem repeat.

When references have a single copy of the 72bp repeat and the sample has two copies of the repeat, reads should pile up to twice the coverage over the single copy 72bp loci as seen in Figure 5A. To test this hypothesis, we added a second 72bp sequence to the shorter plasmid assembly and observed that the reads map without artifact and no evidence of heteroplasmy (Figure 5B).

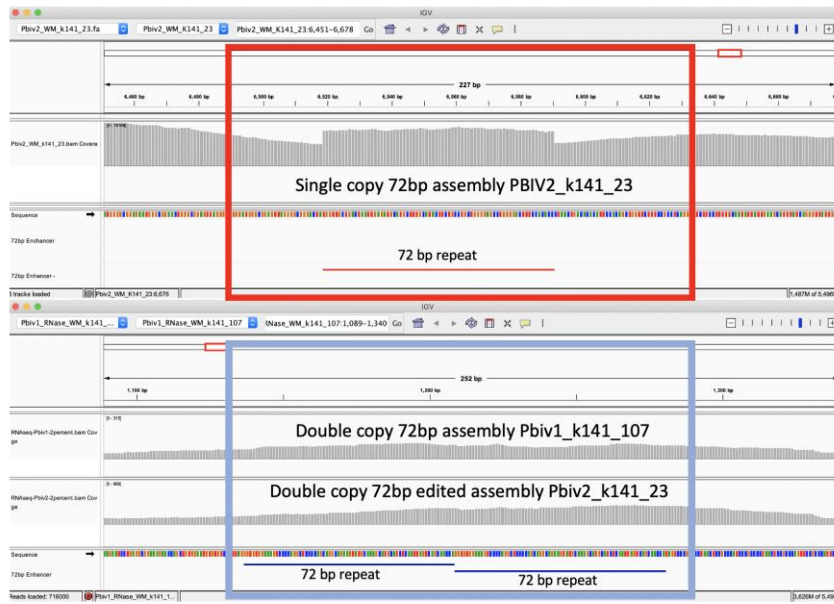


Figure 5B. IGV view of the read coverage over Pbiv2_k141_23 shows a discrete 72bp plateau in coverage (red rectangle). Editing the Pbiv2_k141_23 reference to include 2 copies of the 72bp sequence, and remapping the sequence data to this corrected sequence shows that the coverage over both vectors is more normal with no coverage plateau in Pfizer vial 2.

These data conclude that all Pfizer vectors contain a homoplastic 2 copy 72bp SV40 Enhancer associated with more robust expression and nuclear localization. The initial heteroplastic indel was an artifact of the Megahit assembler and short insert libraries.

To estimate the size of the DNA, the purified vaccines were evaluated on an Agilent Tape Station™ using DNA (genomic DNA screen tapes) and RNA based (high sensitivity RNA tapes) electrophoresis tapes.

Agilent Tape Station™ electrophoresis reveal 7.5 - 11.3 ng/µl of dsDNA compared to the 23.7 - 55.9ng/µl of mRNA detected in each 300µl sample. Qubit™ 3 fluorometry estimated 1-2.8ng/µl of DNA and 21.8ng - 52.8ng/µl of RNA. There is higher fragmentation seen in the DNA electrophoresis. The total RNA levels are less than the anticipated 30ug (100ng/µl) and 100ug (200ng/µl) doses suggesting a loss of yield in DNA and RNA isolation, manufacturing variance or RNA decay with expired lots.

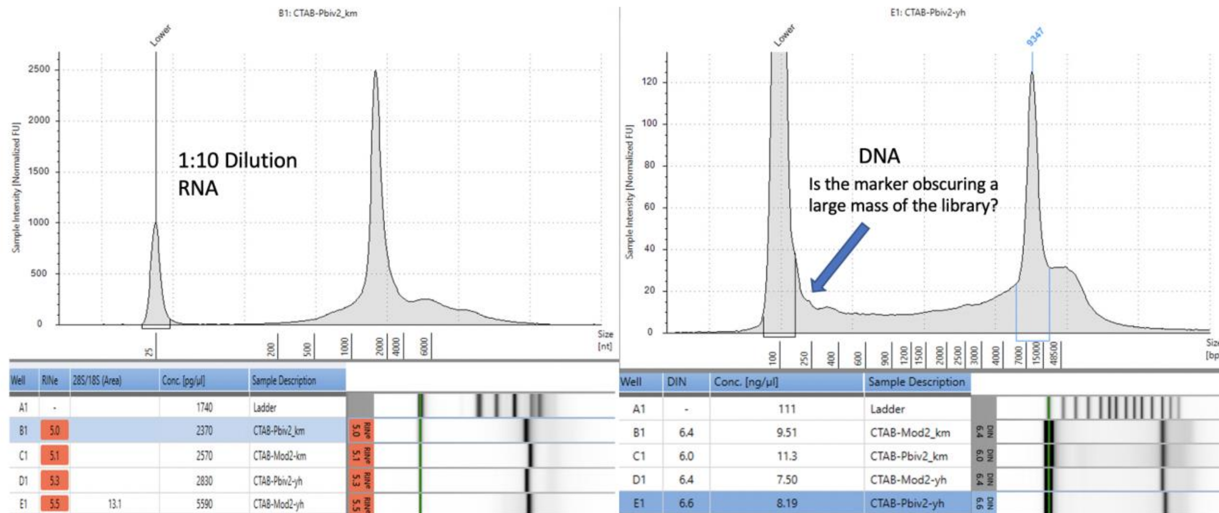
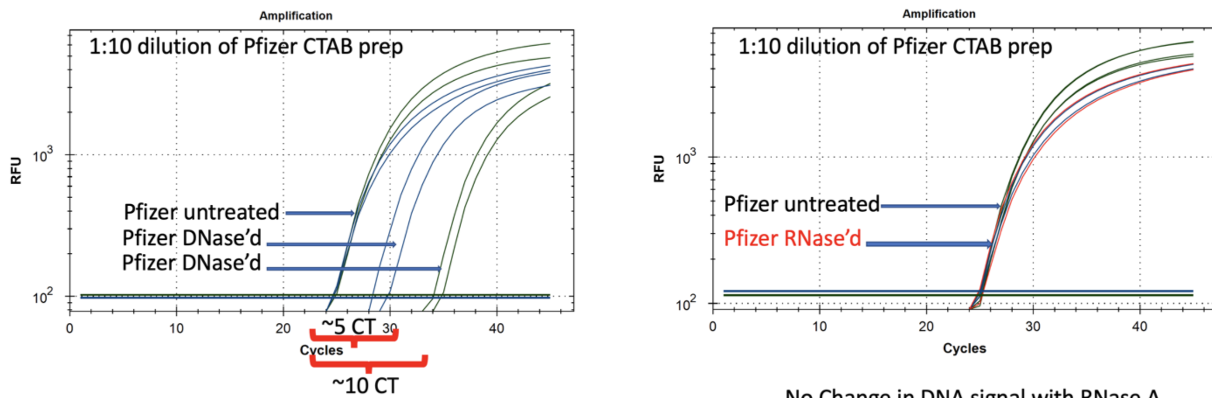


Figure 6. Agilent Tape Station™ electrophoresis demonstrates 23.7ng/µl – 55.9ng/µl of RNA (left). 7.5ng-11.3ng/µl are observed on DNA based Tape Station™. While the DNA electropherogram shows a peak suggestive of a full-length plasmid, this sample is known to have high amounts of N1-methylpseudouridine RNA present. DNA hybrids with N1-methylpseudouridine mRNA may provide enough intercalating dye cross talk to produce a peak. The sizing of the peak on the RNA tape on the left is shorter than expected. This may be the results of N1 methylpseudouridine changing the secondary structure or the mass to charge ratio of the DNA.

Quantitative PCR assays were designed using IDTs Primer Quest software targeting a region in the spike protein that was identical between Moderna and Pfizer spike sequences and a shared sequence in the vectors' origin of replication. This allowed the qPCR and RT-qPCR assessment of the vaccines. qPCR only amplifies DNA while RT-qPCR amplifies both DNA and RNA. Gradient qPCR was utilized to explore conditions where both targets would perform under the same cycling conditions for both RT-qPCR and PCR (gradient PCR data not shown).

Multiplex qPCR targeting Spike (Blue) and Vector Origin (Green)

qPCR Amplifies **ONLY** DNA



5 CT shift on Spike = 32 fold reduction in DNA with DNase I.
10CT shift on Vector = 1000 fold reduction in DNA with DNase I
qPCR does not amplify RNA

Figure 7. qPCR of Pfizer's bivalent vaccine with and without DNase I (left) and RNase A (right). Untreated mRNA demonstrates equal CTs for Spike and Vector assays as expected. Vector is more DNase I sensitive than the Spike suggesting the modRNA may inhibit nuclease activity of DNase I against complementary DNA targets. RNase A treatment doesn't alter the qPCR signal.

Multiplex RT-qPCR targeting Spike (Blue) and Vector Origin (Green)

RT qPCR Amplifies **BOTH** RNA and DNA

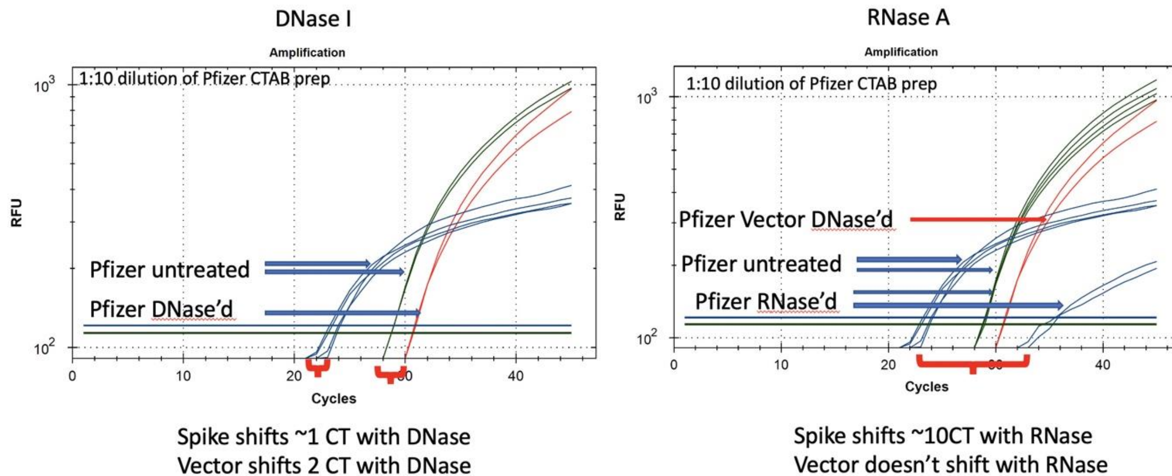


Figure 8. RT-qPCR amplifies both DNA and RNA. The untreated samples show a large CT offset with Pfizer Spike and Vector assays (Left Blue versus Green). This is anticipated as the T7 polymerization should create more mRNA over spike than over the vector. Small 1-2 CT shifts are seen with DNase I treatment. This is expected if the DNA is less than equal concentration of

nucleic acid in RT-PCR. RNase treatment (Right) shows a 10 CT offset but doesn't alter the DNA vector CT.

Pfizer qPCR Results

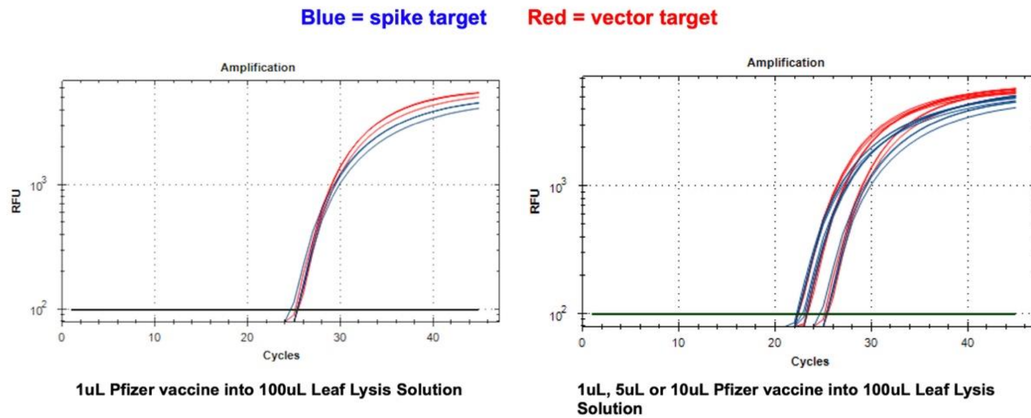


Figure 9. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 24 for both Vector and Spike targets in qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

Pfizer RT-qPCR Results

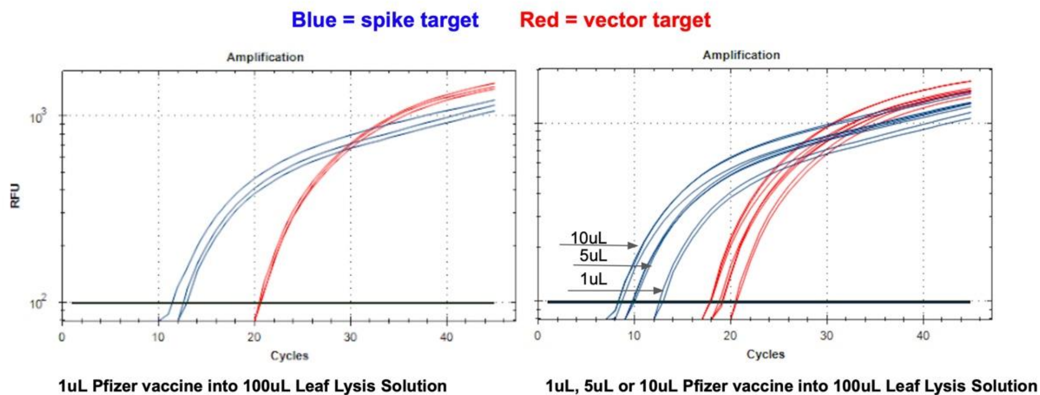


Figure 10. 1µl of the Pfizer bivalent vaccine placed in 100µl Leaf Lysis buffer for an 8 minute boil step delivers a CT of 20 and 12 for both Vector and Spike targets in RT-qPCR (Left). Assay is responsive to 1,5,10µl of input (Right).

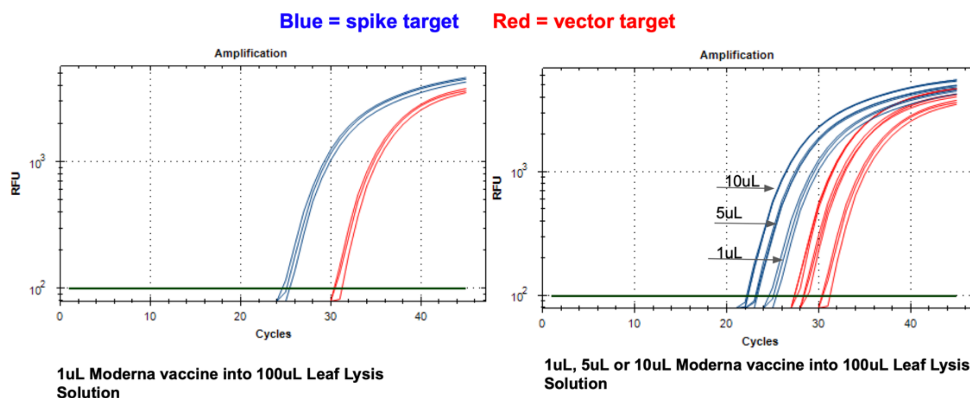


Figure 11. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with qPCR. This needs to be explored further as the assays provide equal CT scores on Pfizers' vaccines and the sequence of the amplicon is identical between the two vector origins. There are 2 mismatches in the spike amplicons between Moderna and Pfizer but none of the mismatches are under a primer or probe. The assay is responsive to 1,5,10µl of direct boil mRNA (Right).

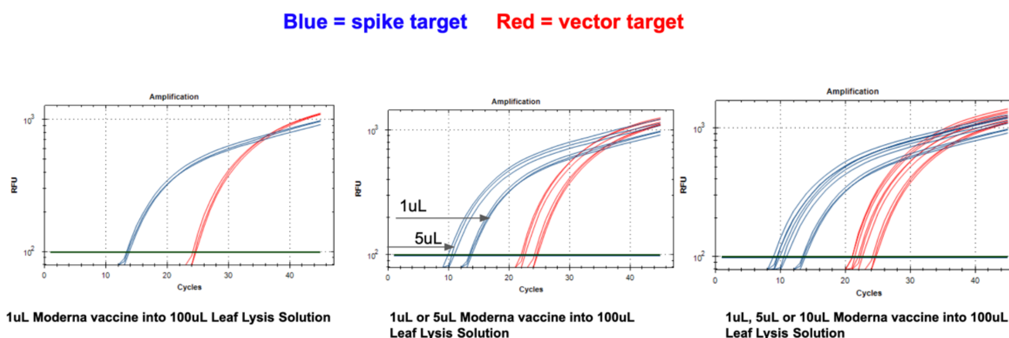


Figure 12. 1µl of the Moderna bivalent vaccine exhibits different CTs values for the spike and the vector targets (Left) with RT-qPCR. The large 10 CT shift between Spike and Vector needs to take into consideration that qPCR control shows a 5 CT offset. The boil preps can tolerate 1-10µl of vaccine (Middle and Right).

	Qubit DNA ng/µl	Qubit RNA ng/µl
Pbiv1	2.81	30.0
Pbiv2	1.47	52.8
Mod1	2.67	21.8
Mod2	1.04	49.0

Table 1. Qubit™ 3 Fluorometry estimates 1.04-2.8 ng/µl of dsDNA in the vaccines and 21.8ng-52.8ng/µl of RNA.

Synthetic templates were synthesized with IDT to build RT-qPCR standard curves to benchmark CTs to the mass of DNA in the reaction. This method uses ideal templates and fails to quantitate DNA molecules smaller than the amplicon size. As expected, this method delivers lower DNA concentration estimates than Qubit™ 3 fluorometry or Agilent Tape Station™. It also represents an ideal environment which doesn't capture the inhibition or primer depletion that can occur when large quantities of mRNA with identical sequence to your DNA target are co-present in a qPCR assay.

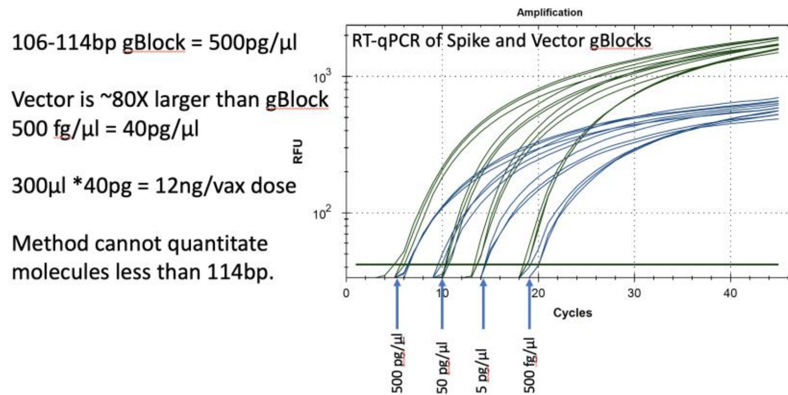


Figure 13. Two gBlocks were synthesized at IDT for Spike and Ori positive control templates used in an RT-qPCR assays. 10-fold serial dilutions were run in triplicate to correlate CT scores with picograms of DNA. The threshold is lowered from 10^2 for review of the background. CT of ~20 = 500fg/RT-qPCR reaction. Since 100bp targets only represent 1/80th of the vector DNA present as a potential contaminant, 500 fg/µl manifests in 40pg/µl of vector DNA. Any DNA that is DNase I treated and is smaller than the amplicon size cannot amplify or be quantitated with this method. This method will under quantitate DNase I treated samples compared to Qubit™ 3 or Agilent Tape Station™.

This work was further validated by testing 8 unopened Pfizer monovalent vaccines with both qPCR and RT-qPCR.

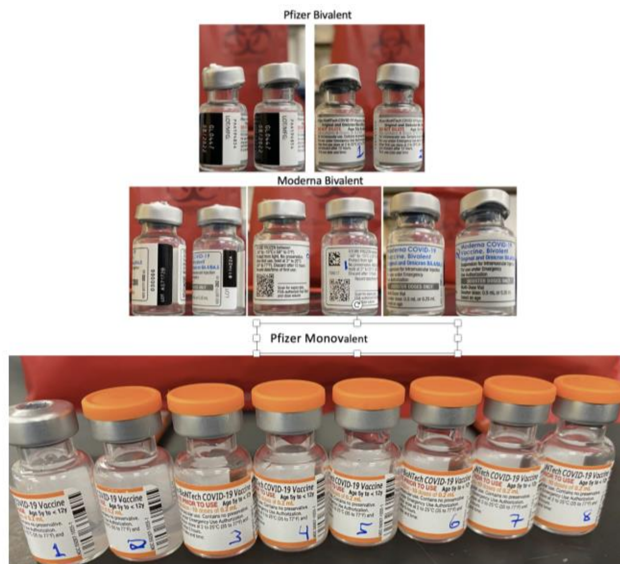


Figure 14. Moderna and Pfizer Bivalent vaccines (Top). 8 Monovalent Pfizer mRNA vaccines. These were unopened but past expiration (Bottom).

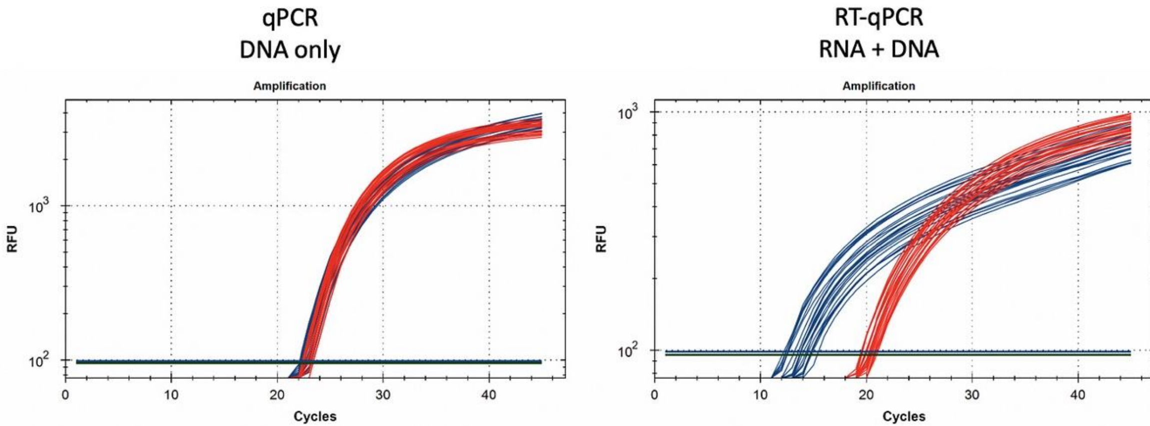


Figure 15. 1µl of vaccine boiled in 100µl of Leaf Lysis buffer was subjected to qPCR (left) and RT-qPCR (right) for Vector (red) and Spike (blue). 8 samples were tested in triplicate.

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 2. CT values for Spike and Vector during qPCR (DNA only). Standard deviation for the 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 and 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 3. CT values for Spike and Vector during RT-qPCR (RNA+DNA). Ratio of RNA:DNA ranges from 43:1 To 161:1. EMA allowable limit is 3030:1. This is 18-70 fold over the EMA limit.

Discussion

Multiple methods highlight high levels of DNA contamination in the both the monovalent and bivalent vaccines. While the Qubit™ 3 and Agilent Tape Station™ differ on their absolute quantification, both methods demonstrate it is orders of magnitude higher than the EMAs limit of 330ng DNA/ 1mg RNA. qPCR and RT-qPCR confirms the relative RNA to DNA ratio. An 11-12 CT offset should be seen between Spike and Vector RT-qPCR signals to represent a 1:3030

contamination limit (2^11.6 = 3100). Instead, we observe much smaller CT offsets (5-7 CTs) when looking at qPCR and RT-qPCR data with these vaccines. It should be noted that Qubit™ 3 and Agilent methods stain all DNA in solution while qPCR measures only amplifiable molecules without DNase I cut sites between the primers. The further apart you space the qPCR primers, the fewer Qubit™ 3 and Agilent detectable molecules will amplify. The primers used in this study are 106bp and 114bp apart, thus any molecules that are DNase I cut below this length will be undercounted with the qPCR methods relative to more general dsDNA measurements from Qubit™ 3 or Agilent Tape Station™.

This also implies that qPCR standard curves using 100% intact synthetic DNA standards will amplify more efficiently and thus undercount the total digested DNA contamination. For example, standard curves with 106-114bp synthetic templates provide CTs under 20 in the picogram range (not low nanogram range) suggesting large portions of the library are smaller than the minimum amplifiable size. Pure standards also do not contain high concentrations of modified mRNA with identical sequence which could serve as a competitive primer sink or inhibitor to qPCR methods.

Alternatively, the Qubit™ 3 and the Agilent Tape Station™ could be inflating the DNA quantification due to intercalating dye cross talk with N1-methylpseudouridine RNA. For this reason, we believe the ratio we observed when these molecules are more scrupulously interrogated with polymerases specific for each template type in qPCR and RT-qPCR is a more relevant metric. The EMA metric is also stated as such a ratio.

This also brings into focus if these EMA limits took into consideration the nature of the DNA contaminants. Replication competent DNA should arguably have a more stringent limit. DNA with mammalian promoters or antibiotic resistance genes may also be of more concern than just random background *E.coli* genomic DNA from a plasmid preparation (Sheng-Fowler et al. 2009). Background *E.coli* DNA was measured with qPCR and had CT over 35.

There has been a healthy debate about the capacity for SARs-CoV-2 to integrate into the human genome(Zhang et al. 2021). This work has inspired questions regarding the capacity for the mRNA vaccines to also genome integrate. Such an event would require LINE-1 driven reverse transcription of the mRNA into DNA as described by Alden *et al*. (Alden et al. 2022). dsDNA contamination of sequence encoding the spike protein wouldn't require LINE-1 for Reverse Transcription and the presence of an SV40 nuclear localization signal in Pfizer's vaccine vector would further increase the odds of integration. This work does not present evidence of genome integration but does underscore that LINE-1 activity is not required given the dsDNA levels in these vaccines. The nuclear localization of these vectors should also be verified.

Prior sequencing of the monovalent vaccines from Jeong *et al.* only published the consensus sequence (Dae-Eun Jeong 2021). The raw reads for this project are not available and should be scrutinized for the presence of vector sequence.

Given these vaccines exceed the EMA limits (330ng/mg DNA/RNA) with the Qubit™ 3 and Agilent data and these data also exceed the FDA limit (10ng/dose) with the more conservative qPCR standard curves, we should revisit the lipopolysaccharide (LPS) levels. Plasmid contamination from *E.coli* preps are often co-contaminated with LPS. Endotoxins contamination can lead to anaphylaxis upon injection (Zheng et al. 2021).

A limitation of this study is the unknown provenance of the vaccine vials under study. These vials were sent to us anonymously in the mail without cold packs. RNA is known to degrade faster than DNA and it is possible poor storage could result in faster degradation of RNA than DNA. RNA as a molecule is very stable but in the presence of metals and heat or background ubiquitous RNases, it can degrade very quickly. All of the vaccines in this study are past the expiration date listed on the vial suggesting more work is required to understand the DNA to RNA ratios in fresh lots. The publication of these qPCR primers may assist in surveying additional lots with more controlled supply chains. Studies evaluating vaccine longevity in breast milk or plasma may benefit from vector DNA surveillance as this sequence is unique to the vaccine and may persist longer than mRNA.

While the sequencing delivered full coverage of the plasmid backbones, it is customary to assemble plasmids from DNase I fragmented libraries. These methods have not discerned the ratio of linear versus circular DNA in the vials. While plasmid DNA is more competent and stable, linear DNA may have higher genome integration risks.

The intercalating dyes used in the Qubit™ 3 and Agilent systems are known to have low fluorescent cross talk with DNA and RNA but it is unknown to what degree N1-methylpseudouridine alters the specificity of these intercalating dyes. As a result, we have relied on the CT offsets between RT-qPCR and qPCR with the vector and spike sequence as the best relative assessment of the EMA ratio-metric regulation. These qPCR and RT-qPCR reagents may be useful in tracking these contaminants in vaccines, blood banks or patient tissues in the future.

Methods

Purifying the mRNA from the LNPs

LiDs/SPRI purification

100µl of each vial was sampled (1/3rd to 1/5th of a dose)

- 5µl of 2% LiDs was added to 100µl of Vaccine to dissolve LNPs
- 100µl of 100% Isopropanol
- 233µl of Ampure (Beckman Genomics)
- 25µl of 25mM MgCl2 (New England Biolabs)

Samples were tip mixed 10X and incubated for 5 minutes for magnetic bead binding. Magnetic Beads were separated on a 96-well magnet plate for 10 minutes and washed twice with 200µl of 80% EtOH. The beads were left to air dry for 3 minutes and eluted in 100µl of ddH20. 2µl of eluted sample was run on an Agilent Tape Station™.

CTAB/Chloroform/SPRI purification of Vaccines

Some variability in qPCR performance was noted with our LiDs/SPRI purification method of the vaccines. This left some samples opaque and may represent residual LNPs in the purification. A CTAB/Chloroform/SPRI isolation was optimized to address this and used for further qPCR and Agilent electrophoresis. Briefly, 300µl of Vaccine was added to 500µl of CTAB (MGC solution A in SenSATIVAx MIP purification kit. #420004). The sample was then vortexed and heated for 5 minutes at 37°C. 800µl of chloroform was added, vortexed and spun at 19,000 rpms for 3 minutes. The top 250µl of aqueous phase was collected and added to 250µl of solution B and 1ml of magnetic binding buffer. Samples were vortexed and incubated for 5 minutes and magnetically separated. The supernatant was removed and the beads washed with 70% Ethanol two times. Samples were finally eluted in 300µl of MGC elution buffer.

Simple boil preparation for evaluating vaccine qPCR.

This boil prep process simply takes 1-10µl of the vaccine and dilutes it into a PCR compatible leaf lysis buffer and heats it (Medicinal Genomics part number 420208).

- 65°C for 6 minutes
- 95°C for 2 minutes

Library Construction for Sequencing

50µl of each 100µl sample was converted into RNA-Seq libraries for Illumina sequencing using the NEB NEBNext UltraII Directional RNA library Kit for Illumina (NEB#E7760S).

To enrich for longer insert libraries the fragmentation time was reduced from 15 minutes to 10 minutes and the First strand synthesis time was extended at 42°C to 50 minutes per the long insert recommendations in the protocol.

No Ribo depletion or PolyA enrichment was performed as to provide the most unbiased assessment of all fragments in the library. The library was amplified for 16 cycles according to the manufacturers protocol. A directional library construction method was used to evaluate the single stranded nature of the mRNA. This is an important quality metric in the EMA and TGA disclosure documents as dsRNA (>0.5%) can induce an innate immune response. dsRNA content is often estimated using an ELISA. Directional DNA sequencing offers a more comprehensive method for its estimation and was previously measured and 99.99% in Jeong et al. It is unclear how this may vary lot to lot or within the new manufacturing process for the newer bivalent vaccines.

RNase A treatment of the Vaccines

RNase A cleaves both uracils and cytosines. N1-methylpseudouridine is known to be RNAse-*L* resistant but RNase A will cleave cytosines which still exist in the mRNAs. This leaves predominantly DNA for sequencing. Vaccine mRNA that was previously sequenced and discussed here, was treated at 37°C for 30 minutes with 10µl of 20 Units/µl Monarch RNase A from NEB. The RNase reaction was purified using 1.5X of SenSATIVAx (Medicinal Genomics #420001). Sample were eluted in 20µl ddH20 after DNA purification. 15µl was used for DNA sequencing.

DNase treatment of the vaccines

50µl of CTAB purified vaccine was treated at 37°C for 30 minutes with 2µl DNase I and 6µl of DNase I buffer (Grim reefer MGC#420143). 2.5µl of LiDs Lysis buffer was added to stop the DNase reaction. Reactions were purified using 60µl 100% Isopropanol, 140µl Ampure, 15µl MgCl2. Magnetic beads were tip mixed 10 times, left for 5 minutes to incubate, magnetically separated and then washed twice with 80% EtOH.

Whole genome shotgun of RNase'd Vaccines.

15µl of the DNA was converted into sequence ready libraries using Watchmakers Genomics WGS library construction kit. This kit further fragments the DNA to smaller sizes making fragment length in the vaccines difficult to predict.

Qubit™ 3 Fluorometry

Qubit™ 3 fluorometry was performed using Biotum AccuBlue RNA Broad Range kit (#31073) and Biotum AccuGreen High Sensitivity dsDNA Quantitation Kit (#31066) according to the manufacturers instructions.

E.coli qPCR

Medicinal Genomics PathoSEEK™ E.coli Detection assay (#420102) was utilized according to the manufacturers instructions.

qPCR and RT-qPCR Spike Assay

- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Forward
- >AGATGGCCTACCGGTTCA
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Reverse
- >TCAGGCTGTCCTGGATCTT
- MedGen-Moderna_Pfizer_Janssen_Vax-Spike_Probe
- >/56-FAM/CGAGAACCA/ZEN/GAAGCTGATCGCCAA/3IABkFQ/

qPCR and RT-qPCR Vector Origin Assay

- MedGen_Vax-vector_Ori_Forward
- >CTACATACCTCGCTCTGCTAATC
- MedGen_Vax-vector_Ori_Reverse
- GCGCCTTATCCGGTAACTATC
- MedGen_Vax-vector_Ori_Probe
- /5HEX/AAGACACGA/ZEN/CTTATCGCCACTGGC/3IABkFQ/

Elute primer to 100uM according to IDT instructions.

Make 50X primer-probe mix.

1. 25µl 100uM Forward Primer
2. 25µl 100uM Reverse Primer
3. 12.5µl 100uM Probe
4. 37.5µl nuclease free ddH20.

Use 15µl of this mixture in the **qPCR master mix** setup seen below. (0.5µl primer/probe per reaction)

Use 10µl of this mixture in the **RT-qPCR master mix** setup seen below.

Medicinal Genomics Master Mix kits used

1. https://store.medicinalgenomics.com/qPCR-Master-Kit-v3-200-rxns
2. https://store.medicinalgenomics.com/pathoseek-rt-qpcr-master-kit

Reaction setup for 30 reactions of qPCR

- 114µl Enzyme Mix (green tube)
- 24µl Reaction Buffer (blue tube)
- 246µl nuclease free ddH20
- 15µl of Primer-Probe set Spike
- 15µl of Primer-Probe set Ori

Use 13.8µl of above MasterMix and 5µl of purified sample (1µl Vax DNA/RNA + 4µl ddH20 if CT <15)

Reaction setup for 34 reactions of RT-qPCR

- 200µl Enzyme mix
- 96µl nuclease free ddH20
- 20µl RNase Inhibitor (purple tube)
- 4µl DTT (green tube)
- 10µl Primer-Probe set Spike
- 10µl Primer-Probe set Ori

10µl of MasterMix and 1µl of Vax DNA/RNA

Medicinal Genomics MIP DNA Purification Kit used

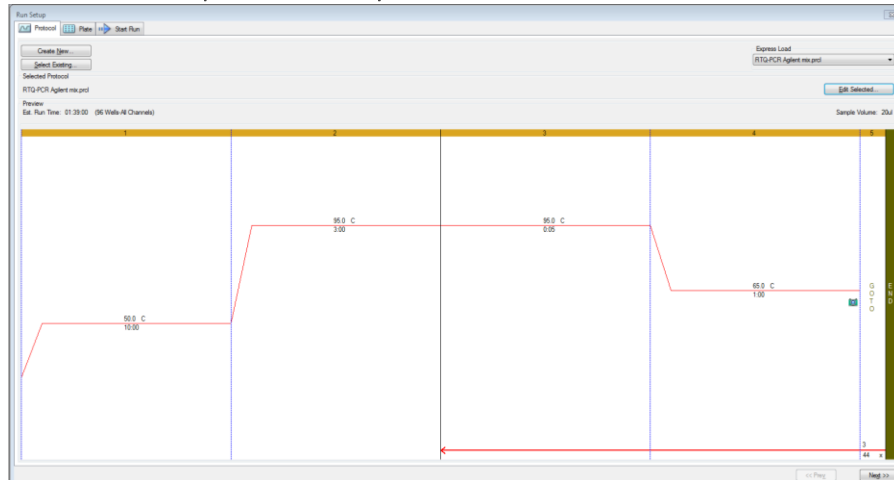
1. https://store.medicinalgenomics.com/SenSATIVAx-DNA-Extraction-Kit-200-reactions_2

he CTAB/Chloroform/SPRI based DNA/RNA isolation methods are described above.

Cycling conditions

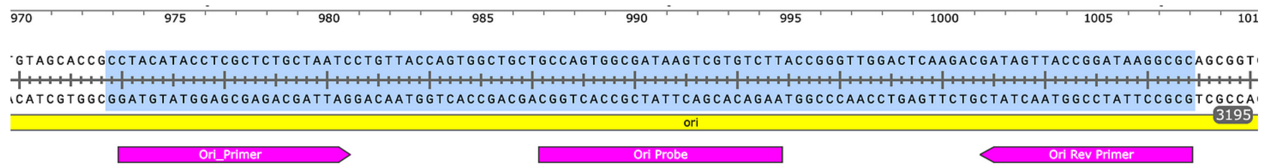
These conditions work for both qPCR and RT-qPCR. Note: The 50°C RT step can be skipped with qPCR. The MGC qPCR MasterMix kits used have a hot start enzyme which are unaffected by this 50°C step. For the sake of controlling RNA to DNA comparisons, we have put qPCR and RT-qPCR assays on the same plate and run the below program with the RT step included for all samples.

Cycling Conditions used for qPCR and RT-qPCR

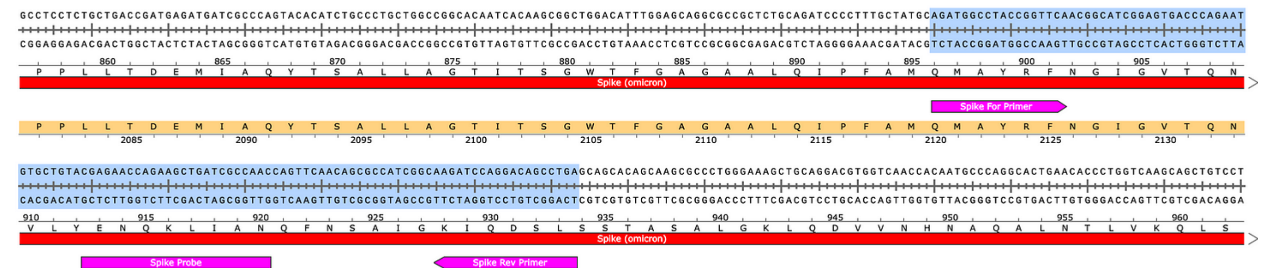


Sequences of amplicons for gBlock Positive Controls. Ori = 106bp, Spike = 114bp.

Ori target



Spike target



Sequencing Data

Raw Illumina Reads RNA-seq

- Pfizer Bivalent Vial 1 Forward reads
- Pfizer Bivalent Vial 1 Reverse reads
- Pfizer Bivalent Vial 2 Forward reads
- Pfizer Bivalent Vial 2 Reverse reads
- Moderna Vial 1 Forward reads
- Moderna Vial 1 Reverse reads
- Moderna Vial 2 Forward reads
- Moderna Vial 2 Reverse reads

Read files are run through sha256 (Hash and stash) and etched onto the DASH blockchain. The sha256 hash of the read file is spent into the OP_RETURN of an immutable ledger. If the hash of the file doesn't match the hash in these transactions, the file has been tampered with.

- Pfizer Vial 1 Forward hash
- Pfizer Vial 1 Reverse hash
- Pfizer Vial 2 Forward hash
- Pfizer Vial 2 Reverse hash
- Moderna Vial 1 Forward hash
- Moderna Vial 1 Reverse hash
- Moderna Vial 2 Forward hash
- Moderna Vial 2 Reverse hash

Megahit Assemblies

- Pfizer Vial 1
- Pfizer Vial 2
- Moderna Vial 1
- Moderna Vial 2

Illumina Reads mapped back to Megahit Assemblies

- Pfizer Vial 1 BAM File. Index File
- Pfizer Vial 2 BAM File. Index File
- Moderna Vial 1 BAM File. Index File
- Moderna Vial 2 BAM File. Index File

Q30 Filtered Illumina Reads (use these for transcriptional error rate estimates)

FastQ-Filter download: usage> fastq-filter -e 0.001 -o output.fastq input.fastq

- Pfizer bivalent Vial 1 Forward Reads
- Pfizer bivalent Vial 1 Reverse Reads
- Pfizer bivalent Vial 2 Forward Reads
- Pfizer bivalent Vial 2 Reverse Reads
- Moderna bivalent Vial 1 Forward Reads
- Moderna bivalent Vial 1 Reverse Reads
- Moderna bivalent Vial 2 Forward Reads
- Moderna bivalent Vial 2 Reverse Reads

Q30 BAM files. Q30 Reads mapped against Megahit assemblies

- Pfizer Vial 1 q30-BAM file. Index File
- Pfizer Vial 2 q30-BAM file. Index File
- Moderna Vial 1 q30-BAM file. Index File
- Moderna Vial 2 q30-BAM file. Index File

IGVtools error by base on q30 reads

Fields = Position in contig, Positive stand (+)A, +C, +G, +T, +N, +Deletion, +Insertion, Negative strand -A, -C, -G, -T, -N, -Deletion, -Insertion

- Moderna Vial 1

- Moderna Vial 2
- Pfizer Vial 1
- Pfizer Vial 2

Analysis pipeline

Reads were demultiplexed and processed with

- Trimgalore - Removes Illumina Sequencing adaptors.
- Megahit- assembles reads into contigs.
- Megahit for SARs-CoV-2
- Samtools- generates BAM files for viewing in IGV.
- Samtools stats used to calculate outie reads.
- BWA-mem- Short read mapper used to align reads back to the assembled references.
- SnapGene software- (www.snapgene.com)- Used to visualize and annotate expression vectors
- IGV- Integrated Genome Viewer used to visualize Illumina sequencing reads.

RNase Treated Libraries-BAM files

contig specific BAM files were created using samtools

```
samtools view -h input.bam contig_name -O BAM > contig.bam; samtools index contig.bam;
```

Samtools stats run on a each contig in each assembly.

```
for out_prefix in `ls *.sort.bam | perl -pe "s/.sort.bam//"`; do mkdir -p ${out_prefix}-samtools-stats; for contig in `samtools view -H ${out_prefix}.sort.bam  | grep "^@SQ"  | cut -f 2 | perl -pe "s/SN\://"`; do echo "Now calculating stats for ${contig}/$out_prefix..."; samtools stats ${out_prefix}.sort.bam $contig > ${out_prefix}-samtools-stats/${contig}-samtools-stats.txt; done; done
```

- Pbiv1_RNase_WM_k141_107.fa
- Pbiv1_RNase_WM_k141_107.bam
- Pbiv1_RNase_WM_k141_107.bam.bai
- Pbiv2_RNase_WM_k141_23.fa
- Pbiv2_RNase_WM_k141_23.bam

- Pbiv2_RNase_WM_k141_23.bam.bai

Author contributions
KJM- constructed the sequencing libraries, designed the qPCR assays, ran Qubit™ 3s and Agilent Tape Station™ and performed the analysis, drafted the manuscript.
YH-Optimized DNA isolations, Tape Station™ and qPCR results.
SM, LTK- assisted in demultiplexing and trimming the reads and assembly troubleshooting

Conflicts of interest- Authors of this paper are employees of Medicinal Genomics which manufacturers some of the qPCR and DNA isolation kits used in this study.

Acknowledgements.
We'd like to thank Lei Zhang for organizing the sequencing outsourcing. We'd like to thank Jessica Rose, Sabine Hazan, Jikkyleaks, Pathogenetics, Steve Massey, Valentine Bruttel, Lynn Fynn, Sasha Letypova, David Wiseman and Pharmacoconuts for helpful comments on this topic.

References

Alden M, Olofsson Falla F, Yang D, Barghouth M, Luan C, Rasmussen M, De Marinis Y. 2022. Intracellular Reverse Transcription of Pfizer BioNTech COVID-19 mRNA Vaccine BNT162b2 In Vitro in Human Liver Cell Line. *Curr Issues Mol Biol* **44**: 1115-1126.

Bansal S, Perincheri S, Fleming T, Poulson C, Tiffany B, Bremner RM, Mohanakumar T. 2021. Cutting Edge: Circulating Exosomes with COVID Spike Protein Are Induced by BNT162b2 (Pfizer-BioNTech) Vaccination prior to Development of Antibodies: A Novel Mechanism for Immune Activation by mRNA Vaccines. *J Immunol* **207**: 2405-2410.

Castruita JAS, Schneider UV, Mollerup S, Leineweber TD, Weis N, Bukh J, Pedersen MS, Westh H. 2023. SARS-CoV-2 spike mRNA vaccine sequences circulate in blood up to 28 days after COVID-19 vaccination. *APMIS* **131**: 128-132.

Dae-Eun Jeong MM, Karen Artiles, Orkan Ilbay, Andrew Fire*, Kari Nadeau, Helen Park, Brooke Betts, Scott Boyd, Ramona Hoh, and Massa Shoura*. 2021. Assemblies of putative SARS-CoV2-spike-encoding mRNA sequences for vaccines BNT-162b2 and mRNA-1273. *GitHub*.

Dean DA, Dean BS, Muller S, Smith LC. 1999. Sequence requirements for plasmid nuclear import. *Exp Cell Res* **253**: 713-722.

Hanna N, Heffes-Doon A, Lin X, Manzano De Mejia C, Botros B, Gurzenda E, Nayak A. 2022. Detection of Messenger RNA COVID-19 Vaccines in Human Breast Milk. *JAMA Pediatr* **176**: 1268-1270.

Josephson F. 2020-11-19. Rapporteur's Rolling Review assessment report. *Committee for Medicinal Products for Human Use* **EMEA/H/C/005735/RR**.

Moreau P, Hen R, Wasylyk B, Everett R, Gaub MP, Chambon P. 1981. The SV40 72 base repair repeat has a striking effect on gene expression both in SV40 and other chimeric recombinants. *Nucleic acids research* **9**: 6047-6068.

Nance KD, Meier JL. 2021. Modifications in an Emergency: The Role of N1-Methylpseudouridine in COVID-19 Vaccines. *ACS Cent Sci* **7**: 748-756.

Sheng-Fowler L, Lewis AM, Jr., Peden K. 2009. Issues associated with residual cell-substrate DNA in viral vaccines. *Biologicals* **37**: 190-195.

Ulrich-Lewis JT, Draves KE, Roe K, O'Connor MA, Clark EA, Fuller DH. 2022. STING Is Required in Conventional Dendritic Cells for DNA Vaccine Induction of Type I T Helper Cell-Dependent Antibody Responses. *Front Immunol* **13**: 861710.

Zhang L, Richards A, Barrasa MI, Hughes SH, Young RA, Jaenisch R. 2021. Reverse-transcribed SARS-CoV-2 RNA can integrate into the genome of cultured human cells and can be expressed in patient-derived tissues. *Proceedings of the National Academy of Sciences of the United States of America* **118**.

Zheng Q, Wang T, Zhu X, Tian X, Zhong C, Chang G, Ran G, Xie Y, Zhao B, Zhu L et al. 2021. Low endotoxin E. coli strain-derived plasmids reduce rAAV vector-mediated immune responses both in vitro and in vivo. *Mol Ther Methods Clin Dev* **22**: 293-303.