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VIA ELECTRONIC FILING

February 10, 2021

Division of Dockets Management
Department of Health and Human Services
Food and Drug Administration
Commissioner Stephen M. Hahn, M.D.
5630 Fishers Lane
Rm. 1061
Rockville, MD 20852

Re: *Citizen Petition and Petition for Administrative Stay of Action (Docket Number: FDA-2020-P-2225)*

Dear Commissioner Hahn,

Attached is an amended reply to the FDA's December 11, 2020 response to Dr. Sin Lee's Citizen Petition and petition for administrative stay of action regarding efficacy endpoints of the Phase III trial of BNT162 and COVID-19 cases being confirmed by Sanger sequencing.

This demands your careful attention and Dr. Lee looks forward to receiving a timely response. Dr. Lee is available to answer questions and provide any relevant additional information.

Very truly yours,

/s/ Aaron Siri

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Rebuttal to the December 11, 2020 Food and Drug Administration's Response to Citizen Petition and Petition for Administrative Stay of Action (Docket Number: FDA-2020-P-2225)

February 10, 2021

Executive Summary

The Center for Biologics Evaluation and Research of the Food and Drug Administration (FDA), has made an arbitrary and capricious decision to deny the requests of the Citizen's Petition and of the Petition for Stay of Action in the matter of efficacy evaluation of the Pfizer's mRNA vaccine for COVID-19 prevention. The FDA knew or should have known:

1) In the Pfizer's vaccine Phase 3 clinical trial, the definition of the 162 confirmed COVID-19 cases in the placebo-receiving participants, which were used as the endpoints to support a 95% vaccine efficacy claim, was the presence of a mild non-specific clinical symptom plus a SARS-CoV-2 NAAT (nucleic acid amplification test)-positive result. Since the mild clinical symptom is non-specific, the pivotal criterion to qualify as a case of COVID-19 in the clinical trial was the NAAT result.
2) The NAAT test (or tests) used for detection of SARS-CoV-2 in the clinical trial was not cleared or approved by the FDA. It was allowed to be used for preliminary detection of SARS-CoV-2 in clinical specimens under Emergency Use Authorization only. These PCR-based tests are known to generate false-positive and false-negative test results.
3) Sanger sequencing-based NAAT is an established irrefutable molecular biology technology for verification of target nucleic acid and SARS-CoV-2 RNA in clinical specimens.
4) Sanger sequencing can confirm the 162 samples as true-positive for SARS-CoV-2, which were used to support the 95% efficacy claim for the Pfizer vaccine, or can re-classify some of these 162 cases as false-positive to revise the percentage of vaccine efficacy.

The FDA rejected the Petitioner's request to re-test the residues of the 162 positive samples by Sanger sequencing, a procedure that could have been accomplished in 2-5 days, for result confirmation to raise public confidence in a newly introduced messenger RNA vaccine, which has no safety track record. The FDA denying consumers the opportunity of having a validated effective and safe vaccine for prevention of COVID-19 is unacceptable.

The Rebuttal

The December 11, 2020 letter signed by Dr. Peter Marks, Director, Center for Biologics Evaluation and Research on behalf of the Food and Drug Administration (FDA) and addressed to Attorney Aaron Siri in response to Dr. Sin Hang Lee's citizen petition dated November 23, 2020 (the CP) and citizen petition for administrative stay of action dated November 25, 2020 relating to the Phase 3 trial of the BNT162b vaccine [the Pfizer vaccine] to prevent the infection by novel coronavirus SARS-CoV-2 (COVID-19) (hereafter referred to as The Letter) [1] must be challenged with a rebuttal for the following reasons.

1. Dr. Peter Marks announced "*I'm the FDA point person on COVID-19 vaccines. We'll make sure they're safe and effective*." This announcement was published in the newspaper USA TODAY on October 27, 2020. [2] In this announcement, Dr. Marks pledged "*to do our duty to the best of our ability, independently and without conflicts of interest, and we will be transparent about FDA decisions*." Dr. Marks recognized "*Trust means everything. Trust in vaccines facilitated the incredible positive impact that vaccination had on public health in reducing illness and death*

over the past century. In the middle of a global pandemic, it is precisely a safe and effective COVID-19 vaccine that will help bring life back to normal if people are willing to receive the vaccine because they have confidence in it. Therefore, it is critical to be open and transparent about the process that the U.S. Food and Drug Administration will follow to help make safe and effective COVID-19 vaccines available." Dr. Marks made a promise to the public that there would be "*Careful evaluation and no rushing"* and stated further *"this process will not be rushed. There will be no shortcuts in developing the relevant phase 3 efficacy results."* [2]

However, the Letter denying the petition and stay shows that the FDA has not conducted an adequate evaluation of the Pfizer vaccine's efficacy, especially concerning issues about the accuracy of RT-qPCR testing of SARS-CoV-2 in clinical specimens. The FDA has misled the public. The key misleading statements are analyzed below point-by-point according to the sequence of their presentation in the Letter but under the following four categories for the convenience of the readers.

A. Cherry-picking to eviscerate the guidance for issuance of an EUA for a COVID-19 vaccine
B. Knowingly promoting inaccurate PCR tests for SARS-CoV-2
C. Finding excuses for using PCR tests with high false-positive rates for this vaccine trial
D. Glossing over potential risks of an mRNA vaccine while concealing its true efficacy

A. CHERRY-PICKING TO EVISCERATE THE GUIDANCE FOR ISSUANCE OF AN EUA FOR A COVID-19 VACCINE

2. Under **II. B. Emergency Use Authorization of the Letter** the FDA omitted some essential elements from the "Emergency Use Authorization for Vaccines to Prevent COVID-19 Guidance for Industry-October 2020" (October 2020 Guidance) [3] in quoting this Guidance as the basis for rejection of Dr. Lee's Citizen Petitions as follows.

"Although EUAs are governed under a different statutory framework than a Biologics License Application (BLA), FDA has made clear that issuance of an EUA for a COVID-19 vaccine would require that the vaccine demonstrated clear and compelling safety and efficacy in a large, well-designed phase 3 clinical trial. In the guidance document Emergency Use Authorization for Vaccines to Prevent COVID-19 (October 2020 Guidance), FDA has provided recommendations that describe key information that would support issuance of an EUA for a vaccine to prevent COVID-19.[12] In the October 2020 Guidance, FDA explained that, in the case of such investigational vaccines, any assessment regarding an EUA will be made on a case-by-case basis considering the target population, the characteristics of the product, the preclinical and human clinical study data on the product, and the totality of the available scientific evidence relevant to the product.[13] FDA has also stated, in the October 2020 Guidance, that for a COVID-19 vaccine for which there is adequate manufacturing information to ensure its quality and consistency, issuance of an EUA would require a determination by FDA that the vaccine's benefits outweigh its risks based on data from at least one well-designed Phase 3 clinical trial that demonstrates the vaccine's safety and efficacy in a clear and compelling manner.[14] A Phase 3 trial of a vaccine is generally a large clinical trial in which a large number of people are assigned to receive the investigational vaccine or a control. In general, in Phase 3 trials that are designed to show whether a vaccine is effective, neither people receiving the vaccine nor those assessing the outcome know who received the vaccine or the comparator."

However, in this quotation of the October 2020 Guidance as the legal basis for granting EUA to the Pfizer vaccine, the FDA has omitted the pivotal parts of the Guidance. The omitted part

is: under **III. CRITERIA AND CONSIDERATIONS FOR THE ISSUANCE OF AN EUA FOR A COVID-19 VACCINE,** the October 2020 Guidance states the following:

"On February 4, 2020, pursuant to section 564(b)(1)(C) of the FD&C Act (21 U.S.C. 360bbb3(b)(1)(C)), the Secretary of HHS determined that there is a public health emergency that has a significant potential to affect national security or the health and security of United States citizens living abroad, and that involves the virus that causes COVID-19. On the basis of such determination, on March 27, 2020, the Secretary then declared that circumstances exist justifying the authorization of emergency use of drugs and biological products during the COVID-19 pandemic, pursuant to section 564(b)(1) of the FD&C Act (21 U.S.C. 360bbb-3(b)(1)).[3]Based on this declaration and determination, FDA may issue an EUA after FDA has determined that the following statutory requirements are met (section 564 of the FD&C Act (21 U.S.C. 360bbb-3))(Ref. 3):

• The chemical, biological, radiological, or nuclear (CBRN) agent referred to in the March 27,
2020 EUA declaration by the Secretary of HHS (SARS-CoV-2) can cause a serious or life-threatening disease or condition.

• Based on the totality of scientific evidence available, including data from adequate and well-controlled trials, if available, it is reasonable to believe that the product may be effective to prevent, diagnose, or treat such serious or life-threatening disease or condition that can be caused by SARS-CoV-2.

• The known and potential benefits of the product, when used to diagnose, prevent, or treat the identified serious or life-threatening disease or condition, outweigh the known and potential risks of the product.

• There is no adequate, approved, and available alternative to the product for diagnosing, preventing, or treating the disease or condition.

In the case of investigational vaccines being developed for the prevention of COVID-19, any assessment regarding an EUA will be made on a case by case basis considering the target population, the characteristics of the product, the preclinical and human clinical study data on the product, and the totality of the available scientific evidence relevant to the product.

C. Safety and Effectiveness Information
The EUA request should include the following safety and effectiveness information, which will inform FDA's determination regarding the product's benefit-risk profile:

1. Bioassays for assessment of clinical endpoints

The diagnostic bioassays that were used to assess study endpoints of clinical studies supportive of the EUA request should be identified. FDA expects that the standard operating procedures (SOPs) and validation reports for the final assay methods, and a list of all laboratories where the clinical samples have been tested, will be submitted to support the EUA request."

3. The Letter shows that the FDA has ignored the statutory requirements in issuing an EUA to the Pfizer vaccine without considering the "*totality of scientific evidence available, including data from adequate and well-controlled trials, if available, it is reasonable to believe that the product may be effective to prevent, diagnose, or treat such serious or life-threatening disease or condition that can be caused by SARS-CoV-2*." because the analysis of the primary outcomes was

based on 170 confirmed cases of COVID-19, including 162 cases in the placebo arm and 8 cases in the BNT162b2 vaccine arm. These 170 cases were trial participants developing any one of the mild symptoms, including fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; or vomiting. Since none of these mild symptoms is specific of COVID-19 or serious or life-threatening disease or condition, evaluation of these clinical endpoints alone does NOT make it "*reasonable to believe that the product may be effective to prevent, diagnose, or treat such serious or life-threatening disease or condition that can be caused by SARS-CoV-2*." Therefore, the statutory requirements for EUA of the Pfizer vaccine have not been met by selecting participants with mild symptoms for vaccine efficacy evaluation.

4. The Letter shows that the FDA has allowed the vaccine manufacturer and the FDA to completely depend on confirmation of COVID-19 cases without serious or life-threatening disease or condition as endpoints, but by relying on a positive RT-qPCR testing result alone. In addition, both the vaccine manufacturer and the FDA failed to evaluate the "*totality of scientific evidence available, including data from adequate and well-controlled trials, if available, it is reasonable to believe that the product may be effective to prevent, diagnose, or treat"* any condition that can be caused by SARS-CoV-2. Failure to evaluate the totality of scientific evidence available, including data from adequate and well-controlled trials, is the major flaw of the FDA in granting EUA to the Pfizer vaccine in violation of its own October 2020 Guidance. The totality of scientific evidence available includes evidence of faulty RT-qPCR tests being used to qualify COVID-19 cases as endpoints for Pfizer vaccine efficacy evaluation, as claimed in the Citizen's Petitions, which was not reviewed by the FDA. This issue will be discussed further below.

5. The Letter totally omitted the section of "***Bioassays for assessment of clinical endpoints***" as required for EUA of COVID-19 vaccines by the October 2020 Guidance. This requirement states "*The diagnostic bioassays that were used to assess study endpoints of clinical studies supportive of the EUA request should be identified. FDA expects that the standard operating procedures (SOPs) and validation reports for the final assay methods, and a list of all laboratories where the clinical samples have been tested, will be submitted to support the EUA request.*" This was perhaps an intentional omission because no data of the diagnostic bioassays that were used to assess study endpoints of clinical studies supportive of the EUA request were presented at the open meeting or published for transparency.

6. The Letter states "*A Phase 3 trial of a vaccine is generally a large clinical trial in which a large number of people are assigned to receive the investigational vaccine or a control. In general, in Phase 3 trials that are designed to show whether a vaccine is effective, neither people receiving the vaccine nor those assessing the outcome know who received the vaccine or the comparator.*" Therefore, it is reasonable to expect that unblinding between participants receiving vaccine and participants receiving saline placebo did not occur in the Pfizer vaccine Phase 3 trial. This is especially important in view of the Pfizer's report as follows. [4]

> *"Pfizer reported safety data for 5,664 people ages 18 to 64 and 1,816 people ages 65 to 85 who received one dose. In the younger group, 38% reported fatigue afterward, while 35% reported headache and 16% had chills. Eleven percent or fewer suffered joint pain, diarrhea or chills. The side effects percentages were lower among the older age group.*
>
> *After the second dose, 36% of trial participants aged 18 to 64 reported fatigue, while 28% reported a headache and 18% reported muscle pain. Again, the data were blinded between placebo and the vaccine candidate.*

Most side effects after the second dose were mild to moderate, but some participants did experience severe or grade 4 side effects that could be life-threatening or disabling, according to the presentation. There were more severe side effects after the second dose as compared with the first dose, even though a smaller number of participants—1,682—were in that group."

So, based on this report after the first dose of the Pfizer vaccine, 38% of the participants had fatigue, 35% a headache, 16% chills, and up-to 11% joint pain, diarrhea or chills. In other words, about all participants receiving vaccine injection experienced some form of vaccine reactions, which would not have occurred among participants receiving sterile normal saline placebo injection.

Since all trial participants were instructed that "*During the 7 days following each vaccination, potential COVID-19 symptoms that overlap with specific systemic events (ie, fever, chills, new or increased muscle pain, diarrhea, vomiting) should not trigger a potential COVID-19 illness visit…*" (8.13 of trial protocol),[5] participants receiving vaccine injection would reasonably assume that they had been protected from COVID-19 and might not trigger a potential COVID-19 illness visit even when they developed a fever; new or increased cough; new or increased shortness of breath; chills; new or increased muscle pain; new loss of taste or smell; sore throat; diarrhea; or vomiting because these symptoms overlap with vaccination reactions and may be caused by numerous pathogens other than SARS-CoV-2.

In contrast, the participants receiving saline injection knew that they were not injected a vaccine product due to the lack of vaccination reactions and felt "unprotected". As a result, the participants in the placebo group tended to contact the trial manager whenever they felt sick with any of those listed mild symptoms, triggering a potential COVID-19 illness visit, which was to be followed by an RT-qPCR test. Such an unblinding process was built-in in the Pfizer vaccine trial design.

Instead of questioning the vaccine sponsor about such a potential unblinding event that might affect the outcomes of vaccine efficacy evaluation, the FDA wanted the Petitioner to produce evidence to prove that "*use of saline injections biases the reporting of symptoms – much less that this asserted compromise leads to a greater number of false positives*." No evidence is needed to prove that 0.5 mL of sterile normal saline is an innocuous material when injected intramuscularly into a healthy person. For the past century, students interested in health care science worldwide have been injecting 0.5 mL of sterile normal saline to each other's buttock in their practical educational classes under supervision of a nurse or physician instructor, with no adverse outcomes other than the minor "prick" from the needle. [6] Furthermore, potential unblinding by lack of symptoms following placebo injections was discussed by VRBPAC members at the December 10 meeting regarding the vaccine. It has been discussed in literature published subsequently. It is disingenuous to assert otherwise.

B. KNOWINGLY PROMOTING INACCURATE PCR TESTS FOR SARS-COV-2

7. Under **III. B. 1. Background Regarding Testing Technology and SARS-CoV-2 Testing** of the Letter, the FDA states the following:

   "*FDA agrees that accurate testing is an important part of ensuring the reliability of vaccine trial outcomes. An accurate test helps identify whether the investigational vaccine prevents COVID-19 (or not) by confirming whether study participants are infected with SARS-CoV-2. Indeed, FDA's June 2020 Guidance states that "[d]iagnostic assays used to support the pivotal efficacy*

analysis (e.g., RT-PCR) should be sensitive [25] and accurate for the purpose of confirming infection and should be validated before use."[26]

Nucleic acid-based amplification tests (NAAT), also referred to as PCR tests, are used to show if individuals have active SARS-CoV-2 infection by detecting the virus's genetic material. In PCR testing, a machine located in a laboratory or at a point of care, depending on the test, runs a series of reactions. These reactions first convert the virus's ribonucleic acid (RNA), if present, into deoxyribonucleic acid (DNA) and then amplify it (make millions of copies of the DNA); the test then detects this DNA. By running multiple amplification cycles, a PCR test can sense even low levels of viral genetic material in a patient's sample, so these tests tend to be highly sensitive (especially laboratory PCR tests).

In a Sanger sequencing-based method, dideoxy-nucleotide (ddNTP) chain terminators are used to determine the specific nucleotide sequence of the target nucleic acid. Current Sanger sequencing-based methods are most commonly carried out via a multistep process, which includes not only appropriate sampling and nucleic acid extraction, but also: 1) conventional PCR amplification of the target region; 2) PCR cleanup for removal of unincorporated primers and nucleotides; 3) a sequencing reaction in which the PCR product is used as template for the incorporation of fluorescently labeled dideoxy chain terminators; 4) sequencing reaction cleanup for removal of unincorporated fluorescent dideoxy chain terminators; and 5) simultaneous size dependent separation and nucleic acid sequence determination.

PCR, when used in conjunction with Sanger-based or other sequencing, can detect and identify viral genetic material in a clinical sample. Historically, PCR has been used with reverse transcription to amplify viral RNA to indicate whether there was a positive signal of any suitable genetic material present, and sequencing has been used to confirm the nucleic acid sequence of the amplified genetic material. As PCR technology has evolved, however, PCR testing does not need to be followed by Sanger or other sequencing for purposes of clinical diagnosis. Currently, reverse real-time PCR (RT-PCR) tests can both amplify and confirm the identity of viral genetic material in a single reaction, without a separate sequencing step.[27] *Many of the NAATs for detection of SARS-CoV-2 that FDA has authorized are based on the technology that both amplifies and confirms viral genetic material without the need for an additional sequencing step.*

We have determined there is not scientific merit in requiring the Phase 3 trial for BNT162 or other COVID-19 vaccine candidates to qualify a PCR diagnosis of COVID-19 with Sanger sequencing. Testing used to support the detection of SARS-CoV-2 infection should be sensitive and accurate, and PCR assays can be sufficiently sensitive and accurate without the need for Sanger sequencing.[28]

FDA's current recommendations for SARS-CoV-2 molecular diagnostic tests include that developers confirm the performance of their assay by testing a minimum of 30 positive specimens and 30 negative natural clinical specimens as determined by an authorized assay.[29] *Additionally, the clinical performance data should demonstrate a minimum of 95% positive percent agreement (i.e., sensitivity) and negative percent agreement (i.e., specificity).*[30]
But FDA has not identified any need to require PCR testing for clinical cases to be followed by Sanger based or other sequencing. We believe that clinical diagnoses can be supported following PCR analyses with a positive percent agreement and negative percent agreement greater than or equal to 95%.[31]

The untrue and half-true statements in this "Background Regarding Testing Technology and SARS-CoV-2 Testing" section must be pointed out for the record as follows.

a) The FDA's statement "*Nucleic acid-based amplification tests (NAAT), also referred to as PCR tests, are used to show if individuals have active SARS-CoV-2 infection by detecting the virus's genetic material*" is misleading and deviates from established FDA guidelines. For example, "FDA defines SARS-CoV-2 molecular diagnostic tests as tests that detect SARS-CoV-2 nucleic acids from human specimens." [7] In a document titled "Molecular Diagnostic Template for Commercial Manufacturers" of SARS-CoV-2 test kits, which the FDA also cited as a reference in its Letter, the FDA specifically defines that the measurand of the diagnostic test is the specific nucleic acid sequences from the genome of the SARS-CoV-2. [8]. In another FDA Memorandum titled "*Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products*", the FDA states that Sanger sequencing is used to determine the specific nucleotide sequence of the target nucleic acid. [9]

   The Letter has not cited any existing FDA guidance that recommends relying on using PCR, especially qPCR or RT-qPCR, to determine the specific nucleotide sequence of the target nucleic acid. With no justification, the FDA has created a set of less stringent standards for evaluation of the accuracy of critical diagnostics and the efficacy of vaccines for humans, relative to FDA's standards for Analysis of Food, Feed, Cosmetics and Veterinary Products.

   All nucleic acid tests, including nucleic acid-based amplification test (NAAT), are designed to determine the sequence of nucleotides. NAAT for SARS-CoV-2 means demonstration of a unique unambiguous SARS-CoV-2 genomic nucleotide sequence in a sample derived from a clinical specimen, to be compared with the known reference sequences annotated in the GenBank database for validation. PCR, including RT-qPCR, cannot determine nucleic acid sequences. To equate PCR with NAAT is misleading.

b) FDA's statement in the Letter "*Indeed, FDA's June 2020 Guidance [10] states that "[d]iagnostic assays used to support the pivotal efficacy analysis (e.g., RT-PCR) should be sensitive [25] and accurate for the purpose of confirming infection and should be validated before use.*"[26] is a reasonable statement, but unsupported by the reference cited. The statement cited reference[25], titled "Guidance for Industry and FDA Staff-Statistical Guidance on Reporting Results from Studies Evaluating Diagnostic Tests Document issued on March 13, 2007" [11] to support the declaration *"[d]iagnostic assays used to support the pivotal efficacy analysis (e.g., RT-PCR) should be sensitive [25]*. However, the entire reference[25] [11] does not contain the words of "pivotal efficacy", "RT-PCR", or "PCR" at all. The document is about statistical guidance. PCR, a nucleic acid amplification tool, does not need statistics. The sensitivity of PCR does not depend on statistical analysis. This document does not deal with vaccine pivotal efficacy analysis or PCR. The second cited document[26], which is identified as "June 2020 Guidance at 17" in the Letter, apparently refers to a document titled "Development and Licensure of Vaccines to Prevent COVID-19, Guidance for Industry U.S. Department of Health and Human Services Food and Drug Administration Center for Biologics Evaluation and Research June 2020". [10] However, this June 2020 Guidance does not cite the March 13, 2007 [11] statistical guidance[25] at all. Instead, it emphasizes *"• Diagnostic assays used to support the pivotal efficacy analysis (e.g., RT-PCR) should be sensitive and accurate for the purpose of confirming infection and should be validated before use."* The key question is how to validate an RT-PCR test result. It is obvious that a positive or negative RT-PCR test whose purpose is to determine if a SARS-CoV-2 gene nucleotide sequence is present in a clinical specimen cannot be validated by statistical manipulations.

FDA has never validated any EUA SARS-CoV-2 PCR tests. It has never established a reference standard.

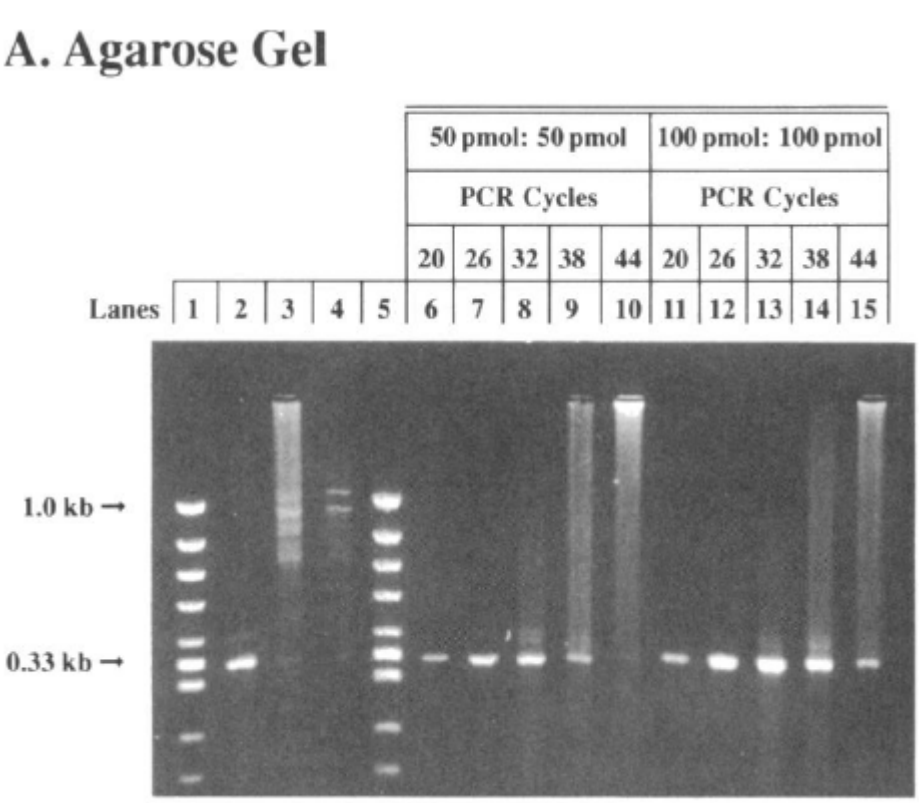
c) In the Letter, the FDA appears to exhibit confusion regarding the terms RT-PCR, RT-qPCR, RT-quantitative PCR (RT-qPCR) or real-time RT-PCR. The FDA June 2020 Guidance [10] specifically mentions under VII. DIAGNOSTIC AND SEROLOGICAL ASSAYS – KEY CONSIDERATIONS that the "*pivotal efficacy analysis (e.g., RT-PCR) should be sensitive and accurate for the purpose of confirming infection and should be validated before use*." According to the National Cancer Institute (NCI) Dictionary of Genetics Terms, "*RT-PCR is a laboratory method used to make many copies of a specific genetic sequence for analysis. It uses an enzyme called reverse transcriptase to change a specific piece of RNA into a matching piece of DNA. This piece of DNA is then amplified (made in large numbers) by another enzyme called DNA polymerase. The amplified DNA copies help tell whether a specific mRNA molecule is being made by a gene. RT-PCR may be used to look for activation of certain genes, which may help diagnose a disease, such as cancer. It may also be used to study the RNA of certain viruses, such as the human immunodeficiency virus (HIV) and the hepatitis C virus, to help diagnose and monitor an infection. Also called reverse transcription-polymerase chain reaction*." [12] The key sentences in this definition are "*RT-PCR is a laboratory method used to make many copies of a specific genetic sequence for analysis. It uses an enzyme called reverse transcriptase to change a specific piece of RNA into a matching piece of DNA. This piece of DNA is then amplified (made in large numbers) by another enzyme called DNA polymerase*." Therefore, RT-PCR is a method to turn a segment of RNA into a segment of matching DNA, which is to be used to generate large numbers of DNA. However, the process of making large numbers of matching DNA pieces in itself does not determine nucleotide sequence. The RT-PCR products need to be validated, especially for vaccine "pivotal efficacy analysis", according to the FDA June 2020 Guidance.[10] As stated above, in a Memorandum titled "Guidelines for the Validation of Analytical Methods for Nucleic Acid Sequence-Based Analysis of Food, Feed, Cosmetics and Veterinary Products", the FDA stated that Sanger sequencing is used to determine the specific nucleotide sequence of the target nucleic acid. [9]

d) In the Letter, FDA's statement "*Historically, PCR has been used with reverse transcription to amplify viral RNA to indicate whether there was a positive signal of any suitable genetic material present, and sequencing has been used to confirm the nucleic acid sequence of the amplified genetic material. As PCR technology has evolved, however, PCR testing does not need to be followed by Sanger or other sequencing for purposes of clinical diagnosis. Currently, reverse real-time PCR (RT-PCR) tests can both amplify and confirm the identity of viral genetic material in a single reaction, without a separate sequencing step.*"[27] is a half-truth. The sentence "*sequencing has been used to confirm the nucleic acid sequence of the amplified genetic material*" is true. But the sentence "*reverse real-time PCR (RT-PCR) tests can both amplify and confirm the identity of viral genetic material in a single reaction, without a separate sequencing step*" while citing an FDA news media pamphlet titled "*A Closer Look at Coronavirus Disease 2019 (COVID-19) Diagnostic Testing*" [13] as supportive reference is totally untrue. The latter news media pamphlet merely summarizes the various types of laboratory tests available for diagnosis of COVID-19. It does not discuss the sensitivity and accuracy of each testing type, let alone presenting any scientific data to support FDA's assertion "*As PCR technology has evolved, however, PCR testing does not need to be followed by Sanger or other sequencing for purposes of clinical diagnosis. Currently, reverse real-time PCR (RT-PCR) tests can both amplify and confirm the identity of viral genetic material in a single reaction, without a separate sequencing step.*"

e) In the Letter, the FDA stated "*We have determined there is not scientific merit in requiring the Phase 3 trial for BNT162 or other COVID-19 vaccine candidates to qualify a PCR diagnosis*

of COVID-19 with Sanger sequencing. Testing used to support the detection of SARS-CoV-2 infection should be sensitive and accurate, and PCR assays can be sufficiently sensitive and accurate without the need for Sanger sequencing.[28]" This statement is an untruth because the entire cited #28 document titled "Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) Immediately in Effect Guidance for Clinical Laboratories, Commercial Manufacturers, and Food and Drug Administration Staff" [14] has not indicated that "*PCR assays can be sufficiently sensitive and accurate without the need for Sanger sequencing*". In contrast, this FDA document emphasizes the need to assure "*publicly available SARS-CoV-2 sequences that can be detected by the proposed molecular assay*". The FDA does not dispute that Sanger sequencing is the method customarily used to determine nucleotide sequences.

f) The FDA's statement "*By running multiple amplification cycles, a PCR test can sense even low levels of viral genetic material in a patient's sample, so these tests tend to be highly sensitive (especially laboratory PCR tests)*" is misleading because that is a half-truth. In fact, by running too many amplification cycles, a PCR test may generate cumulative irrelevant fluorescent signals leading to false-positive results even when there is no target viral genetic material in a patient's sample. It has been known for the past 30 years that excessive cycling may convert PCR products to random-length higher molecular weight fragments even under ideal controlled experimental conditions when no other DNAs are present in the reaction mixture to interfere with the enzymatic process. After 30 cycles of amplification, most of the PCR primers whose base sequences fully match those of their template have been converted into PCR product, and there are no more DNA molecules with fully complementarily matching sequences like the primers and their intended template in the reaction mixture. Under this condition, if amplification cycling continues, the DNA polymerase will be adding nucleotides to the 3'-end of any ssDNA attached to another ssDNA. In other words, by running too many amplification cycles, reaction conditions favor the annealing of the 3'-OH ends of the PCR product to genomic template or to itself after the fully matching primers have been exhausted. As a result, the 3'-OH ends of the PCR product are then extended to higher molecular weight DNA and are randomly terminated during the additional cycles. These random-length products are the likely components of the smear observed with agarose gel electrophoresis. For example, a 1991 study reported that when PCR was used to amplify a target DNA of 0.33kb long, the PCR products were observed as a band after 20-26 cycles of amplification (see Lanes 6, 7, 11, and 12 in the image of agarose gel electrophoresis stained with ethidium bromide below when the gel plate was exposed to ultraviolet light). However, when the cycle numbers increased to 32-44 (Lanes 8, 9, 10, 13, 14 and 15), the PCR products visualized as fluorescent dsDNA/ethidium bromide complexes were no longer tightly identified as one PCR amplicon, but as totally unexpected multiple diffuse high molecular weight products. [15]

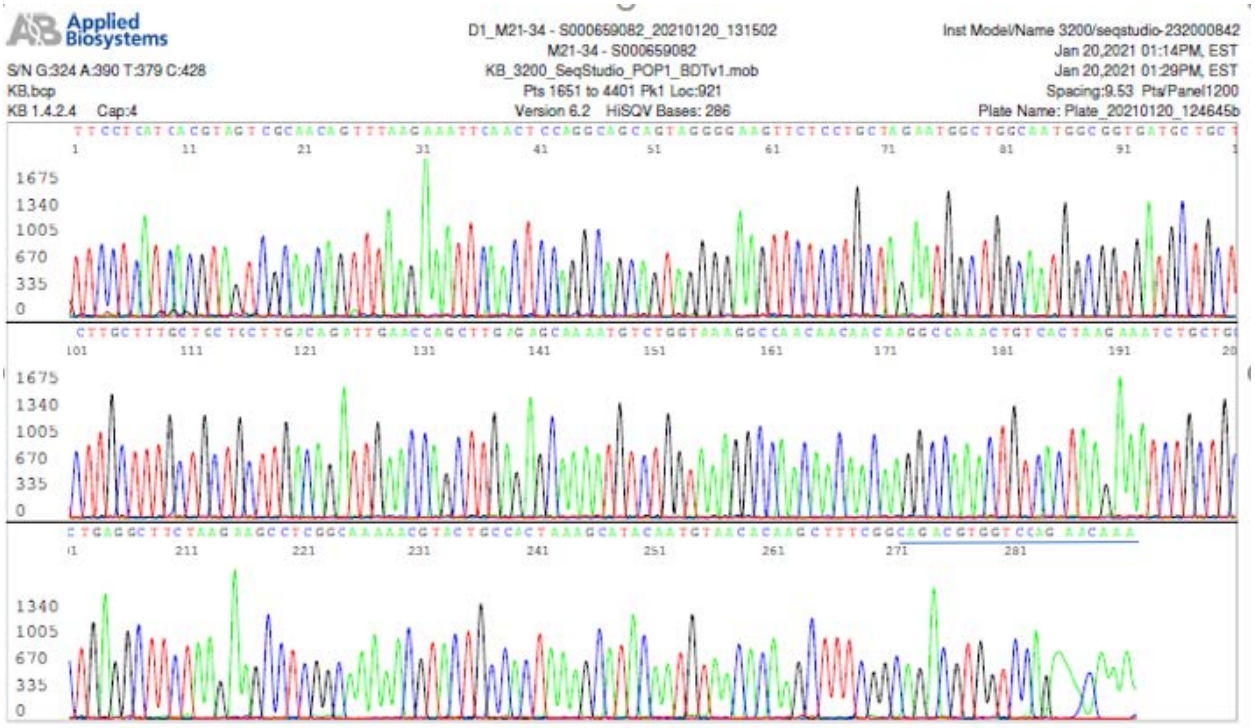


If a fluorometer is used to measure the total signals of fluorescence contained in a test tube without gel electrophoresis, as in the case of qPCR, the fluorometer cannot determine if the total fluorescence is emitted from DNA molecules that would form smears on gel electrophoresis, or is emitted from DNA molecules that would form a specific narrow band on gel electrophoresis. And only the fluorescent molecules that can form a specific band on electrophoresis are likely to be the products of desirable PCR amplification resulting from primer-initiated, template-directed DNA synthesis.

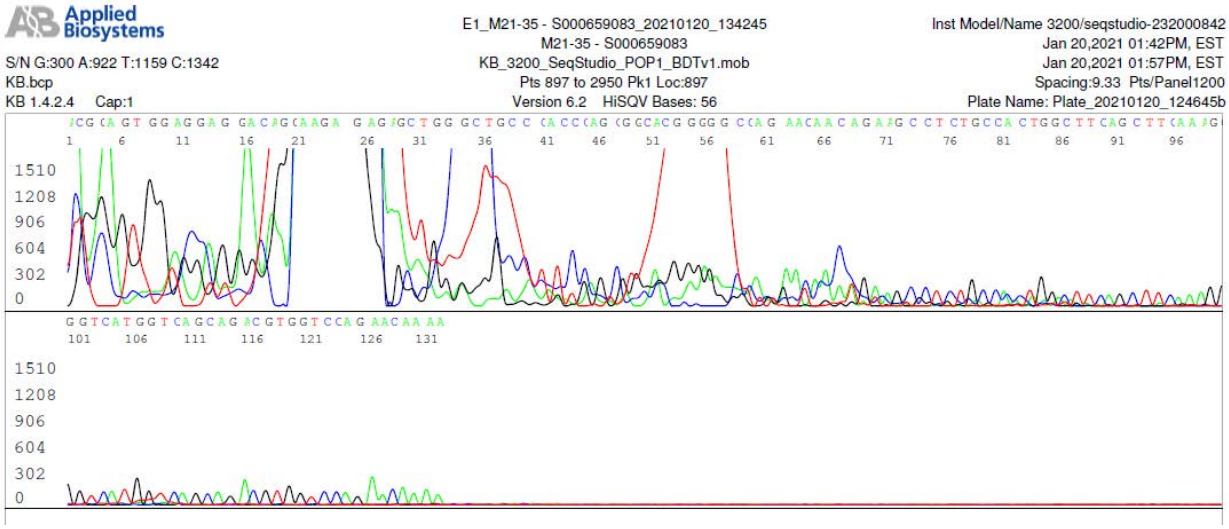
The experiment presented above shows that in the absence of fully matching sequences, any single-stranded DNA (ssDNA) molecules, including PCR primers without fully matching template, will anneal to any ssDNA molecules with partially matching nucleotide sequences at annealing temperature during a PCR cycle. A minimum of only 6 nucleotides matching the sequence of any DNA at the 3′ end of an annealing primer is required to initiate enzymatic primer extension.[16] Random PCR amplification may take place if there is any nontarget DNA with two segments of sequences partially matching those of the primer pair in the reaction mixture to initiate the first PCR cycle. Exponential primer-defined PCR amplification of non-target DNA will proceed after the first PCR cycle is completed. PCR amplification of undesirable DNA in clinical diagnostic work is a well-known phenomenon. For examples, PCR amplification of unintended DNA from *Pusillimonas*, an environmental bacterial species often contaminating patient blood samples, by a pair of specific primers designed for *Borrelia burgdorferi* DNA amplification,[17] PCR amplification of human genomic DNA by PCR primers designed for human papillomavirus L1 gene DNA amplification, [18] and unexpected PCR amplification of Homo sapiens BAC clone RP11-154F14 by the CDC's primers designed for the human RNase P gene [19] have been confirmed by DNA sequencing and reported in peer-reviewed journals. These Sanger sequencing-proven nonspecific PCR products, in the absence of intended target DNA, which were observed during testing of clinical samples, were all due to partial base-matching

between primer and unintended DNA, and provided a mechanism for false positives in PCR-based SARS-CoV-2 testing.

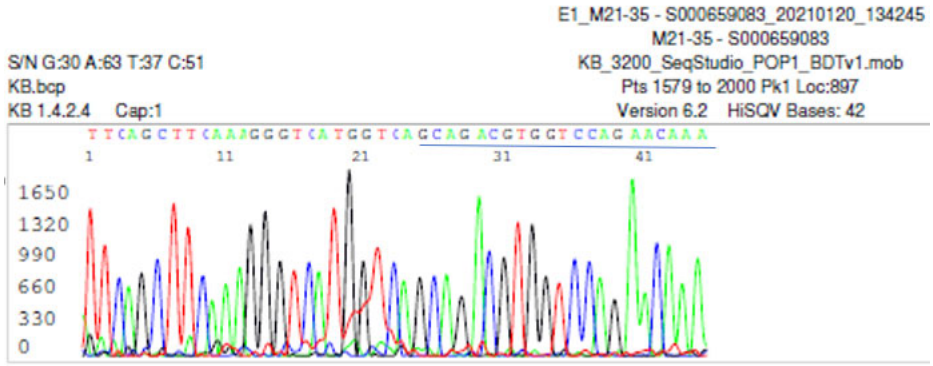
In order to demonstrate the mechanism of how the current EUA RT-qPCR test kits are generating false positives, the Petitioner sequenced 30 human nasopharyngeal swab samples, which had been re-tested at least twice with different EUA test kits and certified as positive for SARS-CoV-2 N gene RNA by a company recommended by the FDA as one of the suppliers of SARS-COV-2 validation panels for new RT-qPCR test developers. [20] The Petitioner designed a pair of 21-base nested PCR primers to amplify a 398-base highly conserved segment of the N gene of SARS-CoV-2 to be used as the template for Sanger sequencing.[19] The sequence of the reverse primer is 5'-TTTGTTCTGGACCACGTCTGC-3'. The results showed that only 16 of the 30 (53%) samples certified to be positive for SARS-CoV-2 N gene based on RT-qPCR were in fact true positives. The complementary sequence of the reverse primer-binding site 3'- GCAGACGTGGTCCAGAACAAA-5' (underlined at the end) is illustrated in the following sequencing electropherogram, showing a unique segment of the SARS-CoV-2 N gene sequence in a true-positive sample (ID# M21-34).



However, parallel sequencing of an adjacent sample ID# M21-35, which was also PCR-positive, showed no SARS-CoV-2 genomic nucleic acid sequence. Instead, the PCR products were composed of various amplified DNA molecules, which were randomly terminated with fluorescent ddNTPs during the Sanger reaction, including one segment of human chromosome 1 DNA that shares part of the sequence of the primer identified above. The raw sequencing electropherogram is pasted below.



Magnification of the readable ending sequence with the 21-base primer DNA (underlined) joining an unintended DNA in sample M21-35 is illustrated below.



Submission of the readable sequence excised from the false-positive sequencing data illustrated in sample M21-35 electropherogram to the GenBank for BLAST analysis elicited return of a report as follows,

Homo sapiens heparan sulfate proteoglycan 2 (HSPG2), RefSeqGene on chromosome 1
Sequence ID: NG_016740.1 Length: 122014 Number of Matches: 1

Score	Expect	Identities	Gaps
84.2 bits(45)	2e-13	45/45(100%)	0/45(0%)

```
Query  1      GCCTCTGCCCCTGGCTTCAGCTTCAAAGGGTCATGGTCAGCAGAC  45
              |||||||||||||||||||||||||||||||||||||||||||||
Sbjct  32609  GCCTCTGCCCCTGGCTTCAGCTTCAAAGGGTCATGGTCAGCAGAC  32653
```

GCAGACGTGGTCCAGAACAAA (CoV primer)
GCCTCTGCCCCTGGCTTCAGCTTCAAAGGGTCATGGTCAGCAGACGTGGTCCAGAACAAA (chimeric)
GCCTCTGCCCCTGGCTTCAGCTTCAAAGGGTCATGGTCAGCAGACTCTGAGGGATGCCA (human)

This experiment confirms that a minimum of only 6 nucleotides (GCAGAC) matching the sequence of any DNA at the 3′ end of an annealing primer is required and sufficient to initiate enzymatic primer extension.[16] In the absence SARS-CoV-2, PCR primers designed for SARS-CoV-2 nucleic acid may amplify any undesirable DNAs with partially matching sequences

from various sources (in this case partially matching nucleotides are typed in red for comparison), leading to false positives if DNA sequencing is not performed to verify the PCR products.

g) In the Letter, the FDA claimed that RT-PCR or RT-qPCR are reliable diagnostic assays for SARS-CoV-2 infection. However, they are not. PCR was invented and perfected as a tool in chemistry for replication of a selected segment of DNA in the test tube to prepare templates for sequence analysis without the need of cloning in the 1980s, as stated in an article by Appenzeller in 1991, before Kary Mullis was awarded a Nobel Prize in 1993 for his discovery.[21] Real-time or quantitative PCR (qPCR) was first described in 1993 to monitor the accumulation of double-stranded DNA (dsDNA) being generated in each PCR using the increase in the fluorescence of ethidium bromide (EtBr) that results from its binding to dsDNA as the PCR products. The kinetics of fluorescence accumulation during thermocycling are directly related to the starting number of DNA copies in the PCR mixture. The basic principle dictates that the fewer cycles necessary to produce a detectable fluorescence, the greater the number of target sequences in the original sample being tested. Results obtained with this approach indicate that a kinetic approach to PCR analysis can quantitate the numbers of a known dsDNA in the mixture, [22] when there are no other interfering DNAs in the PCR mixture. This process is referred to as dye-based qPCR for quantitation of small amounts of target DNA known to exist in a sample. It is widely acknowledged as the most sensitive method to quantify minute amounts of nucleic acids and its applications split into two main types referred to as: relative and absolute quantification. In relative quantification the analyte, often reverse-transcribed mRNA or microRNA, is quantified relative to an endogenous reference. In absolute quantification the targeted nucleic acid (the analyte) is measured relative to a set of standards used to construct a standard curve. [23] qPCR was not designed to determine if a target DNA is present or absent in the sample being tested.

When qPCR is adapted into a “plus/minus” or a “yes/no” assay for the purpose of making the diagnosis of an infectious disease, the dye-based qPCR is converted to a probe-based qPCR. Instead of a free dye like EtBr, a target-specific probe that is an oligonucleotide (ssDNA) of about 25 bases long, complementary to a segment of the target DNA sequence, is introduced into the probe-based qPCR in addition to the PCR primers. The most common probe type is a hydrolysis probe, which incorporates a fluorophore attached to the 5’ end and a quencher attached to the 3’ end of the probe, as the TaqMan® probes commonly used in the SARS-CoV-2 RT-qPCR test kits. [24]

Fluorescence resonance energy transfer (FRET) prevents fluorescence emission of the fluorophore due to proximity of the quencher while the probe is intact. If a target DNA template, among other undesirable DNAs, is present in the PCR mixture, the probe is hydrolyzed during enzymatic primer extension and amplification of the specific sequence to which the primer is bound. The cleavage and degradation of the probe by the 5’-3’ exonuclease activity of the *Taq* polymerase separate the fluorophore from the quencher, allowing fluorescence of the fluorophore and resulting in an amplification-dependent increase in fluorescence. In other words, diagnostic qPCR actually uses the PCR process to test if a DNA/DNA binding (hybridization) has taken place between a set of known oligonucleotides (primers and probe) and a target DNA molecule, which may be present in the sample being tested. It assumes the primers and the probe were all bound to their respective segments of a target ssDNA with fully matching bases before a fluorescence signal was emitted as the result of PCR amplification-dependent degradation of the probe.

In reality, however, this assumption is not always valid. In the nasopharyngeal swab samples taken from patients, there are numerous human cells, bacteria, viruses, plasmids and fungi all of which can contribute nucleic acids, namely DNAs and RNAs, to the sample extract being tested even when there is no SARS-CoV-2 RNA in the specimen. In the absence of fully matching SARS-CoV-2 genomic RNA or cDNA as the preferred target template, the PCR primers and the probe can bind to any partially matched DNA and initiate enzymatic primer extensions and probe degradation. As pointed out above, a minimum of only 6 nucleotides matching the sequence of any DNA at the 3′ end of a primer is required to initiate enzymatic primer extension.[16] PCR amplification may take place if there is a nontarget DNA with two segments of sequences partially matching those of the primer pair in the reaction mixture to initiate the first PCR cycle. Exponential primer-defined PCR amplification of non-target DNA will proceed after the first PCR cycle is completed.

If such an unintended PCR amplification should take place and if the interprimer region of the PCR product also had a sequence matching part of the probe, the probe would attach to the PCR product and undergo hydrolysis by the action of the DNA polymerase during PCR amplification, leading to separation of the fluorophore from its quencher, cycle after cycle, and eventually to a false-positive result. In one DNA/DNA hybridization research study, the authors designed a set of binding partners to a 50-mer oligonucleotide containing complementary stretches from 6 nucleotides (nt) to 21 nt in length. The authors found that stable partial duplexes can form when only 12 bp (12/50) of complementary sequence are present, resulting in the appearance of significant signals from an unintended binding partner, in the absence of the intended fully matched DNA target. [25]

h) In the Letter, the FDA claims *"FDA's current recommendations for SARS-CoV-2 molecular diagnostic tests include that developers confirm the performance of their assay by testing a minimum of 30 positive specimens and 30 negative natural clinical specimens as determined by an authorized assay.[29] Additionally, the clinical performance data should demonstrate a minimum of 95% positive percent agreement (i.e., sensitivity) and negative percent agreement (i.e., specificity).[30] But FDA has not identified any need to require PCR testing for clinical cases to be followed by Sanger-based or other sequencing. We believe that clinical diagnoses can be supported following PCR analyses with a positive percent agreement and negative percent agreement greater than or equal to 95%.[31]"* In the Footnotes, reference [29] is listed as "[29] Id. at 18.", which in turn directs to a statement *" [18] We support the principles of the "3Rs," to reduce, refine, and replace animal use in testing when feasible. We encourage sponsors to consult with us if it they wish to use a non-animal testing method they believe is suitable, adequate, validated, and feasible. We will consider if such an alternative method could be assessed for equivalency to an animal test method."* There is no definition of authorized assay in this statement. So, the FDA has failed to name an assay that he or the FDA considers authorized. In the Footnotes it refers to a document *" [30] Molecular Diagnostic Template for Commercial Manufacturers, July 2020, at 16, https://www.fda.gov/media/135900/download."* However, The FDA Molecular Diagnostic Template for Laboratories [8] states:

*"**B. MEASURAND**: Specific nucleic acid sequences from the genome of the SARS-CoV-2."*

So, according to the FDA the measurand, also known as an object being measured, is specific nucleic acid sequences from the genome of the SARS-CoV-2. PCR cannot determine specific nucleic acid sequences.

In order to cover up the failure to follow FDA's Guidance stated in The Molecular Test Template, the agency simply glossed over it by writing a Footnote ""[31] *When a new test is*

evaluated by comparison to a non-reference standard because no consensus reference standard exists, information on the accuracy of the new test cannot be estimated directly. As a result, performance is demonstrated by the ability of the new test to agree sufficiently with a comparative method. The comparative results are called "positive percent agreement" (which corresponds to sensitivity) and "negative percent agreement" (which corresponds to specificity). The use of this language reflects that the estimates are not of accuracy but of agreement of the new test with the non-reference standard. See Statistical Guidance for Diagnostic Tests, at 11.""

It was astonishing to read a statement from the FDA as late as December 11, 2020 claiming "*no consensus reference standard exists"* on evaluation of the accuracy of the new test for detection of SARS-CoV-2 RNA while hundreds of thousands of full-length genomic sequences have been deposited and published in the GenBank and other global databases. These nucleotide sequences do show that a consensus reference standard, in terms of viral genomic sequences, does exist. The FDA Molecular Diagnostic Template for Laboratories [8] clearly states that the currently used SARS-CoV-2 assay is a real-time RT-PCR test intended for the [**presumptive**] qualitative detection of nucleic acid from the SARS-CoV-2. The FDA Molecular Diagnostic Template for Laboratories [8] further states "*False results can be investigated using an additional EUA RT-PCR assay, and/or Sanger sequencing.*" Since no EUA RT-PCR assays have been validated for their ability to detect specific SARS-CoV-2 nucleic acid sequences, Sanger sequencing is the only reliable technology to detect and verify the consensus reference SARS-CoV-2 RNA.

8. Under **III. B. 2. Petitioner's Argument Regarding HPV Testing** in the Letter the FDA states the following:

"But the recommendations in that guidance have no applicability to the clinical trials for COVID-19 vaccines. The recommendations in the HPV Testing Guidance are for developers of new tests and relate to evaluation of new testing products. Specifically, the guidance recommends that developers of a new HPV test evaluate the ability of the new test to detect the targeted HPV genotypes by comparing the results obtained using the new test to results obtained using either an FDA-approved HPV test that detects the same genotypes, or PCR followed by Sanger sequencing.[33] That is, when developing a new HPV testing technology, one option for manufacturers to evaluate the accuracy of the technology is to confirm whether clinical specimens in fact contain the targeted HPV genotype by comparing the results from the manufacturer's test to the results from Sanger sequencing. The HPV Testing Guidance that Petitioner identifies does not recommend that PCR tests used to diagnose HPV infections in individuals be followed by Sanger sequencing when the tests are used for aiding the diagnosis of an individual's infection.

Therefore, we do not agree that Petitioner's example supports Petitioner's requested action."

In this statement, the FDA tries to justify its double standard used in asymmetrical implementation of drug laws in diagnostics, i.e., one for developers of new tests and one for regulating tests for diagnosis of an individual's infection, or another one for the clinical trials in vaccine development. However, the FDA must use a uniform standard for all because double standards come at the expense of public interest, and are in opposition to FDA regulations. The FDA has not advanced any theoretical or scientific justification for the existence of such double standards. The basis for the Petitioner using the HPV testing guidance of the FDA in supporting the demand for Sanger sequencing confirmation of all SARS-CoV-2 RT-qPCR positive test results is that the HPV guidance has already created the precedent. Specifically, the FDA Guidance on HPV testing advises "*One way to do this is to perform an FDA-approved HPV test*

that detects the same genotypes as your test, or you may perform PCR followed by sequencing of the amplicon (PCR/Sequencing) on your clinical specimens and compare these results to the results of your device."[26] This standard can be applied to any nucleic acid tests for SARS-CoV-2. Since none of the RT-qPCR tests marketed under EUA are FDA-approved, the only acceptable option is to perform a bi-directional Sanger sequencing on the cDNA PCR amplicon.

The FDA should be familiar with its own Guidance documents on NAAT assays for the detection of pathogen genomes, using nucleic acid sequencing for confirmation of test results. Additional examples are illustrated as follows.

a) In a document entitled "Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA - Class II Special Controls Guidance for Industry and FDA Staff, the FDA advises "*Detection of an EV genome in CSF by two different well-characterized and validated nucleic acid amplification tests (NAAT). The NAAT primers pairs should generate amplicons from different genomic regions. One of the NAAT assays should provide sequence information.* ***Bi-directional sequencing should be performed*** *on both strands of the amplicon and the generated sequence should be of an acceptable quality (quality score of 40 or higher as measured by PHRED or similar software packages) and should match the reference or consensus sequence*."[27]

b) In a document entitled "Nucleic Acid-Based In Vitro Diagnostic Devices for the Detection of *Mycobacterium tuberculosis* Complex in Respiratory Specimens - Class II Special Controls Guideline for Industry and Food and Drug Administration Staff, the FDA advises:

"1) Reference Method
Your clinical studies must compare the performance of your device to a composite reference method derived from the results of culture and identification, and direct specimen nucleic acid amplification. More specifically, the composite reference method is defined as testing for MTB-complex by:

i. *Mycobacterial culture and isolate identification* ***AND***
ii. *Direct specimen testing using a FDA cleared or approved nucleic acid amplification based diagnostic device or a non-FDA cleared or approved validated direct nucleic acid PCR* ***amplification test followed by bi-directional sequencing.***

If you use a non-FDA cleared or approved validated direct nucleic acid PCR amplification test followed ***by bi-directional sequencing****, then you must provide additional information regarding the safety and effectiveness of the test to support a determination by the Agency that the test is appropriate for use.*
A positive result is defined as a sample which tests positive by ***either*** *i* ***or*** *ii above.*
A negative result is defined as a sample which tests negative by ***both*** *i* ***and*** *ii above."*[28]

Based on FDA established guidance for molecular diagnostic tests for HPV infection, enterovirus infection and *Mycobacterium tuberculosis* infection, a validated direct nucleic acid PCR amplification test followed by bi-directional sequencing is an acceptable molecular diagnostic method even though these sequencing-based tests are non-FDA cleared or approved. There is no good reason for the FDA to reject validated direct nucleic acid PCR amplification test followed by bi-directional sequencing to perform accurate assays for detection of SARS-CoV-2 RNA in clinical specimens for patient management as well as for vaccine efficacy evaluation since there are no FDA-cleared or FDA-approved tests available. There is no acceptable excuse for the FDA to allow vaccine manufacturers to use presumptive tests only without subsequent confirmatory tests in clinical trials for vaccine efficacy evaluation.

C. FINDING EXCUSES FOR USING PCR TESTS WITH HIGH FALSE-POSITIVE RATES FOR THIS VACCINE TRIAL

9. Under **III. B. 3. Petitioner's Arguments Regarding Vaccine Trial Protocols** in the Letter, the FDA states the following:

a) *"We (FDA) generally agree that "DNA sequencing" after PCR testing is "accepted technology," but we do not agree that this means PCR testing for SARS-CoV-2 must be followed by Sanger-based sequencing for confirmation of infectious agents. That is, for the reasons explained above, we do not agree that PCR testing for SARSCoV-2 must be followed by Sanger-based sequencing in order to diagnose a clinical case of COVID-19,[39] in a clinical trial or otherwise."*

To support its position of relying RT-qPCR testing for diagnosing SARS-CoV-2, the FDA further quoted reference [39] ""*The CDC case definition for COVID-19 notes that confirmatory laboratory evidence is "[d]etection of severe acute respiratory syndrome coronavirus 2 ribonucleic acid (SARS-CoV-2 RNA) in a clinical specimen using a molecular amplification detection test."*"

As elucidated above, in molecular science RT-qPCR assays measure cumulative fluorescent signals as a result of DNA probe hydrolysis, and do not detect severe acute respiratory syndrome coronavirus 2 ribonucleic acid (SARS-CoV-2 RNA). For detection of viral RNA, the FDA guidance as expressed in "Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA" clearly stated " ***Bi-directional sequencing*** *should be performed on both strands of the amplicon and the generated sequence should be of an acceptable quality (quality score of 40 or higher as measured by PHRED or similar software packages) and should match the reference or consensus sequence*."[27] The FDA is trying to create an inferior standard for NAAT detection of viral RNA in order to inflate the number of SARS-CoV-2 infections, by deviation from the established FDA standard for the detection of viral RNA in clinical samples.

b) In response to the Petitions about the WHO guidance on using nucleic acid sequencing for SARS-CoV-2 RNA detection, the FDA claimed "*This WHO guidance does not state that nucleic acid sequencing is critical in all circumstances in order to test accuracy. Rather, it states that the sequencing should be performed "when necessary." Among other things, the guidance contains testing recommendations for when the virus is known to be circulating in a geographic area, and for when the virus is not known to be circulating. When the virus is not known to be circulating in an area, the WHO guidance recommends sequencing as an option. But for areas with established COVID-19 virus circulation, the WHO guidance does not list sequencing as a recommended testing option. We note that this WHO guidance was drafted towards the beginning of the current pandemic, before the development of many of the NAATs that are currently in use. We also note that it does not make any recommendations related to confirming COVID-19 cases in vaccine clinical trials."* In this statement, the FDA agrees that for a geographic area where the SARS-CoV-2 is not known to be circulating, DNA sequencing confirmation is "*an option*", and for areas with established COVID-19 virus circulation, DNA sequencing is not needed, according to the WHO Guidance.

However, in the WHO document cited by the FDA, the phrase "*areas with established COVID-19 virus circulation*" also refers to "*areas where COVID-19 virus is widely spread*".[29] In the Letter, The FDA has not proposed how to define "*established COVID-19 virus circulation*" or "*areas where COVID-19 virus is widely spread'*. However, according to the CDC COVID DATA TRACKER where average daily cases per 100,000 in the last 7 days in different states and the U.S. territories are tabulated and published,[30] as of January 4, 2021, there were no COVID-19

cases in American Samoa, Federated States of Micronesia, Northern Mariana Islands, Palau and the Republic of Marshall Islands. The states with the highest numbers of daily cases of >80/100K are Arizona 121.8, California 97.1, Tennessee 92.5, South Carolina 90.9, Kansas 88.5, Arkansas 88, Oklahoma 83.6, Utah 82.9, West Virginia 82.5, and New York 80.6. The states and the territories with the lowest daily case numbers are Minnesota 29.1, Oregon 27.2, Puerto Rico 23, Vermont 17.5, Hawaii 9.2. Virgin Islands, 7.4, Guam 5.4.

View Historic Case and Death Data | Download Map

Data Table for Average Daily Cases per 100k in Last 7 Days

CDC | Updated: Jan 4 2021 12:16PM | Download Data

State/Territory	Average Daily Cases per 100k in Last 7 Days
Arizona	121.8
California	97.1
Tennessee	92.5
South Carolina	90.9
Kansas	88.5
Arkansas	88
Oklahoma	83.6
Utah	82.9
West Virginia	82.5
New York*	80.6
Rhode Island	79.3
Georgia	78.9
Alabama	76.6
Mississippi	74.8
Connecticut	69.6
Indiana	68.4
Massachusetts	67.1
Louisiana	65.6
North Carolina	65.5
Nevada	65.4
Delaware	65
Kentucky	63.2
Ohio	62.3
Texas	61.5
Florida	60.5
New Hampshire	59.3
Pennsylvania	57.9
New York City*	57.6
New Mexico	57.4
Virginia	52.5
Nebraska	51.9
South Dakota	50.7
Idaho	50.3
New Jersey	49.6
Illinois	47.3
Missouri	45
Alaska	44.4
Maryland	44
Wisconsin	43.3
Iowa	42.5
Wyoming	41.3
Colorado	40.3
Maine	38.8
Montana	34.8
Washington	33.7
District of Columbia	31.6
North Dakota	31.5
Michigan	31.2
Minnesota	29.1
Oregon	27.2
Puerto Rico	23
Vermont	17.5
Hawaii	9.2
Virgin Islands	7.4
Guam	5.4
American Samoa	0
Federated States of Micronesia	0
Northern Mariana Islands	0
Palau	0
Republic of Marshall Islands	0

According to a public document titled "*Pfizer and Biontech announce vaccine candidate against covid-19 achieved success in first interim analysis from phase 3 study*", the Phase 3 clinical trial of BNT162b2 began on July 27, 2020 and enrolled 43,538 participants. The first interim efficacy analysis was conducted on November 8, 2020 by an external, independent Data Monitoring Committee (DMC) from the Phase 3 clinical study. So, the entire Phase 3 study was observed in a period of 105 days from July 27 to November 8, 2020 [31], and eventually data collection extended to November 14, 2020, according to information presented at the VRBPAC meeting.

During these 111 days of observation, there were 170 mild cases of COVID-19, as specified in the study protocol and 10 severe cases of COVID-19, which were observed in the trial. [32]

As a result, the Pfizer vaccine Phase 3 clinical trial was conducted in a population whose average daily COVID-19 cases per 100K was [(170+10) x100,000/43,538]/111=3.7

The number 3.7 is even lower than the lowest number of the average daily COVID-19 cases per 100K observed in any American states and territories (Guam 5.4) except those far-away islands with zero cases. Therefore, it can be readily concluded that the Pfizer vaccine Phase 3 clinical trials were conducted in areas where COVID-19 virus is NOT widely spread, or the virus is NOT known to be circulating in the population, or the trial missed many cases. As the FDA agrees with the WHO Guidance that nucleic acid testing for SARS-Cov-2 in area where the virus is NOT known to be circulating, nucleic acid sequencing should be an option for COVID-19 case confirmation. Refusal to confirm the preliminary positive RT-qPCR test results by nucleic acid sequencing for evaluation of the Pfizer vaccine efficacy is against the standard set by the WHO, which is also promulgated by the FDA itself.

It is well known that when the case prevalence is extremely low in a population, the positive predictive value of an imperfect test may drop down to an unacceptable level. Due to the lack of specificity of symptoms that have been used to trigger RT-qPCR testing for SARS-CoV-2, it is likely that many positive test results are false positives in the Phase 3 trial unless the positive samples are re-tested by Sanger sequencing for confirmation.

The FDA's statement "*We also note that it does not make any recommendations related to confirming COVID-19 cases in vaccine clinical trials*" is of interest because it implies that in its opinion laboratory tests for confirming COVID-19 cases in vaccine clinical trials can be totally deviated from acceptable international standards without transparency.

In the Letter, FDA's statement *"We note that this WHO guidance was drafted towards the beginning of the current pandemic, before the development of many of the NAATs that are currently in use."* implies that the WHO Guidance emphasizing the need for confirmation of SARS-CoV-2 RT-qPCR test results is old and out of date. This is absolutely untrue. As late as December 14, 2020 the WHO issued a new notice titled "*WHO Information Notice for IVD Users Nucleic acid testing (NAT) technologies that use real-time polymerase chain reaction (RT-PCR) for detection of SARS-CoV-2*". In this Notice the WHO states "*WHO has received user feedback on an elevated risk for false SARS-CoV-2 results when testing specimens using RT-PCR reagents on open systems.*" The WHO advises "*Users of RT-PCR reagents should read the IFU carefully to determine if manual adjustment of the PCR positivity threshold is necessary to account for any background noise which may lead to a specimen with a high cycle threshold (Ct) value result being interpreted as a positive result. The design principle of RT-PCR means that for patients with high levels of circulating virus (viral load), relatively few cycles will be needed to detect virus and so the Ct value will be low. Conversely, when specimens return a high Ct value, it means that many cycles were required to detect virus. In some circumstances, the distinction between background noise and actual presence of the target virus is difficult to ascertain. Thus, the IFU will state how to interpret specimens at or near the limit for PCR positivity. In some cases, the IFU will state that the cut-off should be manually adjusted to ensure that specimens with high Ct values are not incorrectly assigned SARS-CoV-2 detected (sic) due to background noise.*" [33]

A more recent document titled "Genomic sequencing of SARS-CoV-2: a guide to implementation for maximum impact on public health. Geneva: World Health Organization", published on January 8th, 2021, further confirms the position of the WHO on the role of gene sequencing by

declaring that "*SARS-CoV-2 gene sequencing can be used in many different areas, including improved diagnostics, development of countermeasures, and investigation of disease epidemiology.*" [34] Vaccine is definitely one of the countermeasures.

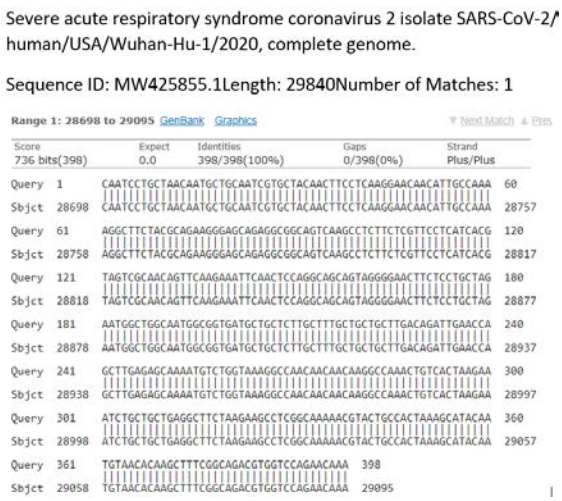
It is irresponsible for the FDA to allow Pfizer to use unverified RT-qPCR test results with a very high Ct value to qualify COVID-19 cases with mild non-specific clinical symptoms in a population with a very low COVID-19 prevalence rate as endpoints in vaccine efficacy evaluation while intentionally misquoting the WHO Guidance to cover up its actions.

c) In the Letter, the FDA disagrees that when FDA issued a letter authorizing emergency use of the CDC 2019-Novel Coronavirus (2019-nCoV) Real Time Reverse Transcriptase (RT)-PCR Diagnostic Panel for the presumptive qualitative detection of nucleic acid from the 2019-nCoV on February 4, 2020, the very word "**presumptive**" carries the meaning of uncertainty in diagnosis. The FDA has allowed laboratories to use "presumptive" molecular tests to diagnose COVID-19 cases based on which public health policies are made for population lockdowns and shutting down schools and businesses, and for artificially inflating the vaccine efficacy in the Pfizer vaccine clinical trials while they know that a simple routine Sanger sequencing of a short segment of SARS-CoV-2 gene RNA can eliminate all false-positive test results. This very action with its resulting negative impacts on the economies of the country should be brought to public attention, and the responsible agencies should be held accountable.

d) In the Letter, the FDA wrote:

Petitioner's assertion: "In addition to false-negative results, these RT-qPCR test kits under EUA also generate false-positive test results." CP at 5.
FDA response: While we agree that no test is 100 percent accurate, this does not support Petitioner's request that FDA require PCR positive cases to be confirmed with Sanger-based sequencing in clinical trials for COVID-19 vaccines.

This statement does not make sense. First, the FDA is wrong in claiming "*no test is 100% accurate*", an attempt to gloss over the false-positive test results often generated by the RT-qPCR test kits permitted to be marketed under EUA by the FDA. The fact is that a correctly performed bi-directional Sanger sequencing of a unique 398-base segment of the SARS-CoV-2 N gene is 100% specific with no possibility of false positivity.[19] Submission of a 398-base sequence to the GenBank for BLAST analysis will induce a report of 100% ID sequence match with that of SARS-CoV-2 with an e-value of 0.0, as shown in the copy of a BLAST report, pasted below. An e-value of 0.0 in a BLAST report is indicative of 100% specificity in molecular identification. Even the FDA cannot refute its validity.


Severe acute respiratory syndrome coronavirus 2 isolate SARS-CoV-2/
human/USA/Wuhan-Hu-1/2020, complete genome.

Sequence ID: MW425855.1Length: 29840Number of Matches: 1

Range 1: 28698 to 29095 GenBank Graphics ▼ Next Match ▲ Prev

Score	Expect	Identities	Gaps	Strand
736 bits(398)	0.0	398/398(100%)	0/398(0%)	Plus/Plus

```
Query  1      CAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAA  60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28698  CAATCCTGCTAACAATGCTGCAATCGTGCTACAACTTCCTCAAGGAACAACATTGCCAAA  28757

Query  61     AGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACG  120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28758  AGGCTTCTACGCAGAAGGGAGCAGAGGCGGCAGTCAAGCCTCTTCTCGTTCCTCATCACG  28817

Query  121    TAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAG  180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28818  TAGTCGCAACAGTTCAAGAAATTCAACTCCAGGCAGCAGTAGGGGAACTTCTCCTGCTAG  28877

Query  181    AATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCA  240
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28878  AATGGCTGGCAATGGCGGTGATGCTGCTCTTGCTTTGCTGCTGCTTGACAGATTGAACCA  28937

Query  241    GCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAA  300
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28938  GCTTGAGAGCAAAATGTCTGGTAAAGGCCAACAACAACAAGGCCAAACTGTCACTAAGAA  28997

Query  301    ATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAA  360
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct  28998  ATCTGCTGCTGAGGCTTCTAAGAAGCCTCGGCAAAAACGTACTGCCACTAAAGCATACAA  29057

Query  361    TGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAA  398
              |||||||||||||||||||||||||||||||||||||||
Sbjct  29058  TGTAACACAAGCTTTCGGCAGACGTGGTCCAGAACAAA  29095
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In essence, the FDA first created an untruth, which is "*no test is 100 percent accurate*", by making a declaration without any scientific basis. Then he in turn claimed that this untruth "*does not support Petitioner's request that FDA require PCR positive cases to be confirmed with Sanger-based sequencing in clinical trials for COVID-19 vaccines*."-because "*no test is 100 percent accurate"*. The purpose may have been to allow using inaccurate RT-qPCR tests to manipulate endpoint statistics for inflating the efficacy of the Pfizer vaccine in prevention of COVID-19.

e) In the Letter, the FDA wrote:

> *While FDA has identified some flaws with some tests, there are many FDA-authorized tests for which FDA has not issued any such alerts (including many tests that use PCR technology, such as Cepheid Xpert Xpress SARS-CoV-2, Roche cobas SARS-CoV-2 real-time RT-PCR test, and Abbott Molecular/RealTime SARS-CoV-2 assay). Moreover, FDA has not stated that samples identified as positive in PCR testing need to be confirmed by Sanger-based sequencing.*

The FDA admitted that some of the FDA-authorized tests have been found to be flawed. However, the FDA has followed a uniform protocol to authorize RT-PCR tests for SARS-CoV-2 RNA detection under EUA. Specifically, the FDA Guidance [8] recommends using a Comparator Method for percent agreement performance calculations for evaluation of new RT-PCR test kits. The FDA requires re-testing with the newly introduced test kit a minimum 30 natural positive clinical specimens and a minimum 30 natural negative clinical specimens for comparisons. Positive percent agreement should be calculated in comparison to an EUA RT-PCR test. Negative result agreement may be calculated in comparison to an EUA RT-PCR test. FDA uses 95% positive and negative agreement as acceptable clinical performance for EUA. Therefore, all EUA RT-PCR test kits on the market should have the same degree of performance accuracy, including bad or good results. If the FDA has found that these EUA RT-PCR test kits, which were supposed to be comparable in performance, are in fact generating test results that are no longer comparable, a reasonable expectation is for the FDA to demand using Sanger sequencing to find out: Which EUA RT-PCR test kits are really at fault? It is irresponsible for the authorized civil servants in the FDA to gloss over such an important issue, which is affecting the health of the citizens and the national economy.

f) In the Letter, the FDA wrote:

> *FDA's COVID-19 Testing Guidance states that all clinical tests should be validated prior to use, and provides recommendations for developers regarding testing that should be performed to demonstrate, in support of an EUA submission, that a SARS-CoV-2 test is validated based*

upon the underlying technological principles of the test.[44] However, FDA does not recommend that clinical results generated from PCR testing should be corroborated with Sanger-based sequencing in order to confirm the clinical performance of a test. Rather, the Molecular Test Template merely states that false results observed during the evaluation of an assay "can be investigated using an additional EUA RT-PCR assay, and/or Sanger sequencing" in order to provide the results of the discordant analysis to FDA.

This is an ambiguous statement designed so that the manufacturers of the test kits do not have to use generally accepted scientific approach to validate the analyte for accurate molecular COVID-19 diagnosis. The FDA continued issuing warnings of faulty EUA RT-PCR tests on the market, as late as January 4, 2021.[35] Manufacturers of newly introduced faulty RT-PCR kits are able to shop around to find the most suitable equally faulty EUA RT-PCR assay for generating a set of comparable test results to meet the FDA acceptable requirement when Sanger sequencing is not used as a standard for result comparisons. FDA regulations require validation of tests according to a gold standard, not to a standard chosen by the test developer.

g) In response to Petitioner's assertion: "According to the FDA guidance on molecular diagnosis of viral infection caused by human papillomavirus (HPV), a conventional PCR detection of genomic DNA followed by Sanger sequencing is recommended", the FDA wrote:

"FDA's recommendations regarding validation are for the testing technology, not clinical results. Petitioner's requested action would not be consistent with FDA's recommendations for clinical testing for HPV when performed by sensitive and accurate PCR tests."

This is a convoluted and confusing statement made by the FDA. The FDA appears to be claiming that validation for testing technology has no relationship with clinical testing or is different from technology used to identify HPV infection. Every medical doctor in practice and every medical student knows that there are no "*clinical results*" of HPV infection of the uterine cervix because HPV infection is asymptomatic. HPV infection is totally dependent on detection of HPV genomic DNA in the cervicovaginal cell specimens. The FDA guidance clearly stated that if the manufacturer of a new test cannot use an FDA-approved test as the comparator for accurate evaluation, they can use a conventional PCR amplicon as the template for a bi-directional Sanger sequencing for test validation. There are no "*FDA's recommendations for clinical testing for HPV when performed by sensitive and accurate PCR tests.*" The latter statement is a fabrication. Since there are no FDA-approved RT-qPCR test kits available for SARS-CoV-2 detection, it is entirely reasonable for the FDA to require Sanger sequencing of a PCR amplicon to verify all RT-qPCR test results, especially for the test results used in vaccine efficacy evaluation, as stated in the October 2020 Guidance as follows- It is all about Clinical Endpoints, Clinical Studies and Clinical Samples to be validated by laboratory testing:

" C. Safety and Effectiveness Information.
The EUA request should include the following safety and effectiveness information, which will inform FDA's determination regarding the product's benefit-risk profile:

1. Bioassays for assessment of clinical endpoints

The diagnostic bioassays that were used to assess study endpoints of clinical studies supportive of the EUA request should be identified. FDA expects that the standard operating procedures (SOPs) and validation reports for the final assay methods, and a list of all laboratories where the clinical samples have been tested, will be submitted to support the EUA request." [3]

For another example, the FDA standard guidance on Clinical Studies for the Detection of viral RNA can be found in a document titled "**Nucleic Acid Amplification Assay for the Detection of Enterovirus RNA - Class II Special Controls Guidance for Industry and FDA Staff".** [27**]**

In this document, the FDA clearly stated "***Bi-directional sequencing should be performed on both strands of the amplicon and the generated sequence should be of an acceptable quality (quality score of 40 or higher as measured by PHRED or similar software packages) and should match the reference or consensus sequence".*** The relevant section is copied and pasted below as evidence.

"8. Clinical studies

You should conduct prospective clinical studies to determine the performance of your device for all the specimen types you claim in your labeling. You should prospectively collect the specimens from individuals with signs and symptoms consistent with clinical suspicion of meningitis or meningoencephalitis. You should describe the protocol of each clinical study (including the inclusion and exclusion criteria, study endpoints, acceptance criteria), and a description of how the studies support the proposed intended use. You should include a sufficient number of samples so that results will be statistically and clinically meaningful. Archived samples may be useful to provide specimens from patients who have symptoms of meningitis, and from whom fresh specimens may not be readily available (e.g., CSF from very young patients). When using the archived specimens, selection protocols should be used to minimize bias, and appropriate archives should be selected. Furthermore, samples should be masked to avoid testing bias. If both fresh and archived frozen samples are tested, we recommend that you analyze the data separately. For archived samples, results should be represented as percent agreement.

We recommend that you assess and compare the performance of your device to a predetermined algorithm that uses composite reference methods. Additionally, your device should also be compared to EV viral culture. The composite reference methods should include laboratory results such as:

1. *Methods that provide clinical evidence consistent with meningitis, for example, laboratory results such as CSF Gram stain, CSF bacterial culture, CSF glucose, CSF-blood glucose ratio, CSF total protein concentration, CSF leukocyte count. Results from additional specimen types, e.g., stool specimen may also be part of the composite reference method.*
2. *Detection of an EV genome in CSF by two different well-characterized and validated nucleic acid amplification tests (NAAT). The NAAT primers pairs should generate amplicons from different genomic regions. One of the NAAT assays should provide sequence information.* ***Bi-directional sequencing should be performed on both strands of the amplicon and the generated sequence should be of an acceptable quality (quality score of 40 or higher as measured by PHRED or similar software packages) and should match the reference or consensus sequence*** *[Ref. 10, 17]."*

h) In the Letter, the FDA wrote:

"While a test sample that is analyzed with a Ct value of 42.9 may find a very small concentration of viral fragments that may be of uncertain clinical significance, Petitioner does not provide any evidence that the Cepheid test being used in Pfizer's (or any other) clinical trial is being used to analyze samples that actually have a Ct value of 42.9. It appears that Petitioner

found the 42.9 number in the Instructions for Use document for the Cepheid test, available on FDA's website.[46] However, the levels cited by Petitioner refer only to the range of concentrations analyzed to establish the test's limit of detection—not to the number of amplification cycles to be used for clinical diagnosis. Therefore, the levels cited by Petitioner do not demonstrate any accuracy problems with the test. The levels cited by Petitioner also do not demonstrate the need for follow-up Sanger-based sequencing."

The FDA's assertion that, "*a test sample that is analyzed with a Ct value of 42.9 may find a very small concentration of viral fragments that may be of uncertain clinical significance*" is untrue because it is not supported by scientific evidence. RT-qPCR tests using Ct 42.9 as cut-off will mostly detect back-ground non-target DNAs. For example, in an extensive research article titled "*SARS-CoV-2 Transmission among Marine Recruits during Quarantine"*, the authors reported that among marine recruits under strict observations and controlled studies SARS-CoV-2 genomes were finally obtained from only 32 of 51 participants (62.7%) who had positive RT-qPCR results for SARS-CoV-2 even when the Ct values used as the positive cut-off was set below 30. [36]

In an article titled "*Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates*", the authors reported that patient samples tested "positive" for SARS-CoV-2 by RT-qPCR at Ct 25 yielded up to 70% virus culture-positive results. At Ct 30, the virus culture-positive rate dropped to 20%. At Ct 35, the value the authors used to report a positive result for RT-qPCR, <3% of cultures were positive. [37] That means a 97% false-positive rate in routine RT-qPCR if virus culture is used as the gold standard for comparison.

Another group of scientists in Australia tested a commercial RT-qPCR test kit and found its positive predictive value for SARS-CoV-2 infection to be only 55.56%. The authors suggested that any positive results derived from one commercial test kit should be confirmed using another nucleic acid test or nucleotide sequencing. [38]

The WHO advised on December 14, 2020 "*Users of RT-PCR reagents should read the IFU (Instructions for User) carefully to determine if manual adjustment of the PCR positivity threshold is necessary to account for any background noise which may lead to a specimen with a high cycle threshold (Ct) value result being interpreted as a positive result. The design principle of RT-PCR means that for patients with high levels of circulating virus (viral load), relatively few cycles will be needed to detect virus and so the Ct value will be low. Conversely, when specimens return a high Ct value, it means that many cycles were required to detect virus. In some circumstances, the distinction between background noise and actual presence of the target virus is difficult to ascertain*." [33]

The FDA defended Cepheid for setting a Ct 42.9 to detect a very small concentration of SARS-CoV-2 in clinical samples without supportive data. The FDA has not performed any experiments to back up this statement on behalf of Cepheid. The FDA wanted the Petitioner to prove that this high "*number of amplification cycles to be used for clinical diagnosis*" was being employed by Pfizer in its Phase 3 trials. This is grossly unreasonable. It was FDA's duty to request or to force the vaccine manufacturers to disclose the details of their laboratory specifications and data for transparency. The FDA is authorized to perform this official function. Citizen petitioners do not have this kind of power.

i) In the Letter, the FDA wrote:

"We agree that no test is 100 percent accurate, and there may be small differences in the analytical performance between different test kits – even kits that are well-validated and reliable. But we do not agree that this justifies Petitioner's requested action – requiring follow-up with Sanger-based sequencing. Tests that are well-validated and reliable may appropriately be used to confirm COVID-19 diagnoses in patients, including study participants."

Where is the evidence that the PCR tests being used are “well validated and reliable”? There is none. According to an official correspondence titled “College of American Pathologists (CAP) Microbiology Committee Perspective: Caution must be used in interpreting the Cycle Threshold (Ct) value”, which was published in Clin Infect Dis. 2020 Aug 12:ciaa1199 and pasted below, the current NAATs granted EUA are not reproducible, even using split samples. Reproducibility using the same kit between labs is much worse. The Petitioner is concerned about a yes-or-no answer to a question: Is there in fact a genomic RNA of SARS-CoV-2, a life-threatening virus, in a person’s respiratory tract specimen when the sample tested is labeled RT-qPCR-positive? The consequences between a positive test result and a negative test result for SARS-CoV-2 detection are not “small differences” as the FDA is trying to lead the public to believe. The truth is that a yes or a positive answer may have serious consequences, including quarantine of the person being tested, putting this person into the same isolation room with COVID-19 patients for dangerous exposure, locking down the schools and businesses in the community with all negative impacts on local economies, and qualifying COVID-19 cases as endpoints for vaccine efficacy evaluation. The differences between yes and no answers are not small both to the individual citizens and to society. The FDA has been placed into a position of responsibility by the taxpayers to make very important decisions. Knowingly choosing not to use the best available technology to perform NAAT for the diagnosis of COVID-19 is inexcusable. In the entire Letter, the FDA has not denied that Sanger sequencing can be 100% accurate with 100% specificity in confirming SARS-Cov-2 detection. The agency refused to use it.

Clinical Infectious Diseases

CORRESPONDENCE

College of American Pathologists (CAP) Microbiology Committee Perspective: Caution Must Be Used in Interpreting the Cycle Threshold (Ct) Value

To the Editor—We read with great interest the article by Magleby and colleagues entitled "Impact of SARS-CoV-2 Viral Load on Risk of Intubation and Mortality Among Hospitalized Patients with Coronavirus Disease 2019" [1]. This article adds to the growing body of work on using the polymerase chain reaction (PCR) cycle threshold (Ct)-value associated with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA detection in clinical specimens as a prognostic indicator and to establish criteria for active infection and transmissibility. Although we recognize the importance of studying laboratory results and their relevance to care of patients with coronavirus disease 2019 (COVID-19), we wish to inform your readers of potential caveats that must be considered when applying published findings regarding Ct-values to their own patients' results.

1) Specimen collection method, specimen source, transport media type and volume, duration from specimen collection to analysis, and days from infection to specimen collection can all impact the amount of viral RNA that could be detectable by an assay, and these variables are reflected in the Ct values.
2) No quantitative SARS-CoV-2 assays have received Emergency Use Authorization (EUA) by the Food and Drug Administration (FDA). Additionally, no international, commutable standardized reference material is currently available, which would be needed for validation of quantitative assays that generate comparable results across manufacturers and laboratories. Although specimens with lower Ct-values generally have more viral RNA than specimens with higher Ct-values, the quantitation and precision associated with those differences in Ct-values have not been determined.
3) Only traditional real-time PCR assays produce a Ct-value. Some diagnostic assays used to detect SARS-CoV-2 RNA use isothermal amplification methods, which do not produce a Ct-value. Other PCR platforms use nested PCR, which is not designed for quantitative interpretation.
4) Ct-values can vary significantly between and within methods. The College of American Pathologists (CAP) recently surveyed more than 700 laboratories using proficiency testing material produced from the same batch (Figure 1). The median Ct-values reported by the instruments for different FDA EUA methods varied by as much as 14 cycles. Within a single test performed on the same instrument, the difference in the median Ct-values for different targets was as high as 3.0 cycles. Finally, within a single gene target for a single method, up to 12.0 cycle differences were seen across all laboratories. The assay and gene target used by Magleby et al, ORF1a detected by the Roche cobas system, differed by approximately 6.0 cycles across all laboratories responding to the survey. Many clinical laboratories are using multiple tests that assess different gene targets for SARS-CoV-2 and are performing testing on different platforms. This adds to the potential variability of Ct-values produced by a single laboratory.

The ongoing shortage of commercial testing reagents presents a major obstacle to conducting large research studies comparing testing platforms. We thus believe that data from the CAP proficiency testing survey

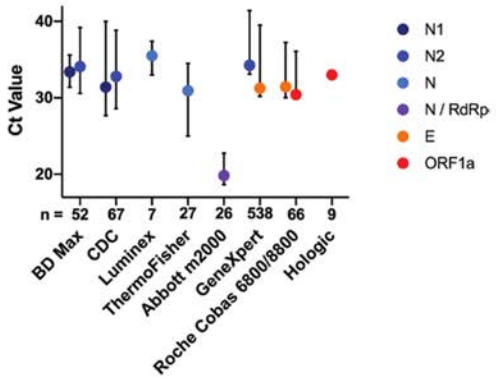


Figure 1. Ct values for gene targets and manufacturers for the same batch of testing material. Median Ct values (*filled circles*) and the range of Ct values from low to high (*whiskers*) are shown. The number of survey respondents using each method is indicated below the *x*-axis. Of note, the material used for the PT Survey did not contain all gene targets in use by commercial assays, and Ct values entered by laboratories under "Miscellaneous" were not incorporated into the data. Data from the users of the Cepheid GeneXpert and GeneXpert Xpress System were combined into a single category for the purposes of this visualization, as both systems employ the same test cartridge and there was likely misreporting between these 2 categories by survey participants. The Hologic category only includes values from the Panther Fusion SARS-CoV-2 assay, as the Hologic Aptima assay does not produce Ct values. Abbreviations: Ct, cycle threshold; PT, proficiency testing; SARS-CoV-2, severe acute respiratory syndrome coronavirus 2.

j) In the Letter the FDA wrote about the Cepheid test kits as follows:

"The alert Petitioner identifies was issued by Diagnostic Laboratory Services Inc., a clinical testing laboratory in Hawaii, and appears to concern the Cepheid GeneXpert testing platform,[49] not the Cepheid Xpert Xpress SARS-CoV-2 assay that is identified in the Pfizer public protocol and with which Petitioner takes issue. In any case, the fact that tests run by one laboratory in Hawaii on Cepheid GeneXpert instruments may have yielded suspect results does not justify the action requested by Petitioner. If sponsors for vaccine clinical trials are using SARS-CoV-2 tests that are well-validated and reliable, there is no scientific reason to require follow-up Sanger-based sequencing."

This statement indicates that the FDA knows that various versions of EUA RT-qPCR test kits, even those made by the same manufacturer for the detection of SARS-CoV-2, such as the Cepheid GeneXpert testing platform and the Cepheid Xpert Xpress SARS-CoV-2 assay, may generate different test results, which may lead to false positives or false negatives. After being informed that a clinical testing laboratory in Hawaii was concerned about Cepheid GeneXpert testing platform generating false-positive result for SARS-CoV-2 in clinical specimens, the FDA engaged in covering up the potential defects of the Cepheid products and the faulty Phase 3 trial protocol for the Pfizer vaccine development instead of requesting the raw data from the vaccine manufacturer for a stringent review, as promised.

k) In the Letter, the FDA further explained away the potential false positives generated by the Cepheid test kits as follows:

"While the study cited by Petitioner found that some samples that were reported as positives using the Cepheid Xpert Xpress SARS-CoV-2 test did not report as positives using the comparison test, the study authors state that "[i]t is difficult to address the question on whether these specimens are true negative samples or low-positive samples with residual viral particles."[51] That is, for the samples that were positive using Cepheid Xpert Xpress SARS-CoV-2 but not the other test, the study authors do not state that the samples were actually negative. Moreover, the study does not make any recommendations regarding the purported need to use follow-up Sanger-based sequencing on results that report to be positive using PCR testing."

Then the FDA further justified the "no need for Sanger sequencing evaluation" for the EUA RT-qPCR tests by stating the following:

"*https://jcm.asm.org/content/58/8/e01136-20. But the Abbott test used in the study, which is compared to the Cepheid Xpert Xpress SARS-CoV-2 test, is the Abbott ID NOW COVID-19, not the Abbott RealTime SARS-CoV-2 assay that is listed in the public protocol identified by Petitioner. We note that, on May 14, 2020, FDA issued a release alerting the public to early data that suggest potential inaccurate results from using the Abbott ID NOW point-of-care test to diagnose COVID-19 because the test may return false negative results. See Coronavirus (COVID-19) Update: FDA Informs Public About Possible Accuracy Concerns with Abbott ID NOW Point-of-Care Test, May 14, 2020, https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fdainforms-public-about-possible-accuracy-concerns-abbott-id-now-point. Therefore, the fact that the Abbott ID NOW COVID-19 and the Cepheid Xpert Xpress SARS-CoV-2 test produced different results is not surprising. The existence of different results from the Abbott ID NOW COVID-19 and the Cepheid Xpert Xpress SARS-CoV-2 test do not support a need for follow-up Sanger-based sequencing from PCR tests that have demonstrated a positive percent agreement and negative percent agreement greater than or equal to 95%, which include the tests identified in the Pfizer public protocol."*

These statements confirm that the FDA contradicts itself. First, the agency claimed the Cepheid Xpert Xpress SARS-CoV-2 test reported was compared with the Abbott ID NOW COVID-19, not

the Abbott RealTime SARS-CoV-2, implying that the latter Abbott test kit might generate better comparative results than the other Abbott kit without citing any evidence to support its claim. However, at the same time, the agency affirmed that all RT-PCR test kits granted EUA by the FDA have demonstrated a positive percent agreement and negative percent agreement greater than or equal to 95%. If the second statement is true, the first claim cannot be valid because the Abbott ID NOW COVID-19 test kit and the Abbott RealTime SARS-CoV-2 would have yielded similar results for comparison with the Cepheid Xpert Xpress SARS-CoV-2 test kit. Since all the test kits cannot generate consistent comparable results, Sanger sequencing is urgently needed as the *de facto* gold standard to find out the true positives among the "presumptive" positive specimens labeled by all EAU RT-qPCR tests. This would reveal the truth. The FDA chose to close its eyes at the expense of public interest. The Abbott RealTime SARS-CoV-2 assay apparently has different sensitivities of detection because the NAAT Detectable Units/mL by this assay can range from 5400 to 2700, according to the data published on the FDA website [39], which are copied and pasted below.

5400	Cepheid	Xpert Xpress SARS-CoV-2 test
5400	Abbott Molecular	Abbott RealTime SARS-CoV-2 assay
2700	Abbott Molecular	Abbott RealTime SARS-CoV-2 assay

Why hasn't the FDA inquired which Abbott assay was in fact used for the Pfizer vaccine Phase 3 clinical trial and how the Abbott assay results were compared with the Cepheid test results?

l) In the Letter, the FDA claimed that the Petitioner did not point out evidence that intramuscular injection of a very small amount of sterile saline in the placebo participants will not cause fever, local redness and swelling, and severe pain, or systemic reactions, which may cause unblinding and bias the reporting system in the Pfizer vaccine Phase 3 trials. The statement of the FDA is copied and pasted as follows.

> *"In addition, Petitioner seems to also claim that follow-up Sanger sequencing is needed to address an asserted bias in the study design. Petitioner asserts that "it is commonly known" that injection of saline (i.e., the placebo) "will not cause fever, local redness and swelling, and severe pain, or systemic reactions." CP at 8. Study participants who receive a placebo therefore "intuitively and reasonably know that they were not injected with a vaccine[.]" CP at 9. Petitioner states that this is relevant to his requested action because, according to Petitioner, this makes placebo participants more likely to report symptoms than vaccine recipients, thereby leading to the use of test kits that will cause "[a] higher number of false-positive test results" among participants in the placebo arm. CP at 9. However, Petitioner has not pointed to any evidence that use of saline injections biases the reporting of symptoms – much less that this asserted compromise leads to a greater number of false positives. Therefore, we do not agree that Petitioner has demonstrated that purported unblinding justifies the action requested.*[52,53,54]

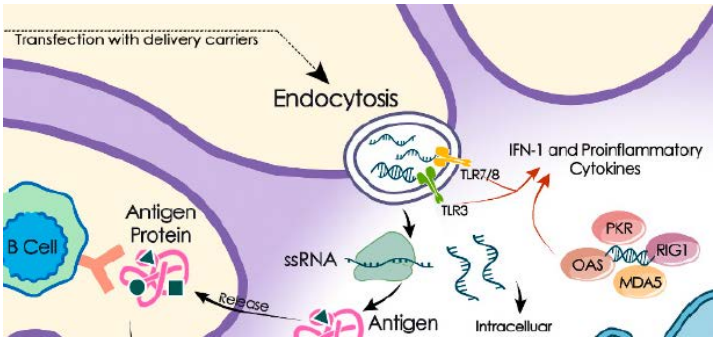
As pointed out above, it is common knowledge that 0.5 mL of sterile normal saline is an innocuous material when injected intramuscularly into a healthy person. For the past century, students interested in health care science worldwide have been injecting 0.5 mL of sterile normal saline to each other's buttock in their practical educational classes under supervision of a nurse or physician instructor, with no adverse outcomes other than the minor "prick" from the needle. [6] Concerns about a breach in blindness or unblinding in the Phase 3 trials due to lack of vaccine-induced symptoms in the participants receiving saline placebo injection, which may lead to more PCR tests among the placebo-receiving participants, have been openly expressed by other scientists. [40]

D. GLOSSING OVER POTENTIAL RISKS OF AN mRNA VACCINE WHILE CONCEALING ITS TRUE EFFICACY

10. Under **III. C. 1. a. Petitioner Has Not Demonstrated Irreparable Injury** in the Letter, the FDA states the following:

> *"Petitioner's claim of injury is too remote. Petitioner asserts that Petitioner will be forced to receive an inadequately vetted vaccine due to mandatory vaccination requirements that purportedly may be issued by entities such as airlines and States. However, the PSA does not seek a stay of any FDA decision that will force any individuals to receive vaccines. FDA does not mandate vaccination. Rather, Petitioner seeks to stay a Phase 3 clinical trial due to asserted problems with the testing protocol but has not demonstrated that the continuation of the trial will cause States, airlines, or any other entity to issue requirements that will in turn cause Petitioner to be vaccinated against Petitioner's will. There are numerous regulatory steps between the conduct of clinical trials and the existence and distribution of a vaccine that is available to the public – much less before any State or other entity makes any potential decisions regarding mandatory vaccination.[56] The continuation of clinical trials, alone, will not cause the asserted harm.[57]*
> *Thus, Petitioner has not demonstrated that the continuation of clinical trials under FDA IND will cause irreparable injury."*

The FDA should know that all vaccines, including the Pfizer mRNA vaccine, have associated potential adverse reactions after injection into healthy humans. The nanoparticles composed of mRNA coated with phospholipids may act as potent toll-like receptor (TLR) agonists after endocytosis, causing local and systemic surges of IFN-1 and proinflammatory cytokines. The molecular focal point of this reaction is illustrated in the diagram cropped from a review article titled "mRNA Vaccine Era-Mechanisms, Drug Platform and Clinical Prospection." [41]



TLR activation may lead to a variety of autoimmune disorders. Some of them are fatal. For example, TLR 7 activation may lead to severe thrombocytopenia in experimental animals, [42] and might have been the cause of death of a 56-year-old physician who developed fatal thrombocytopenia after receiving Pfizer vaccines. [43] Therefore, before introducing such a new vaccine whose active ingredient is synthetic mRNA coated with phospholipids in the form of nanoparticles without safety track record, a high benefit-to-risk ratio must be demonstrated without a reasonable doubt. The FDA is the gatekeeper to maintain this high benefit-to-risk ratio for approval of the Pfizer vaccine. The Petitioner simply requested that the FDA use due diligence to ensure that the preliminary laboratory test results, which the vaccine manufacturer used as the pivotal criteria in qualifying COVID-19 cases as endpoints for vaccine efficacy evaluation, are properly verified. If the vaccine's efficacy is not as high as claimed due to false-positive test results in the Phase 3 clinical trials, the Petitioner and his fellow citizens may be forced by various business operators to take a vaccine with high risk and uncertain benefits in the name of COVID-19 prevention even if there is no government mandate to do so.

In an arbitrary dismissal of the Petitions, the FDA claimed that the Petitioner "*has not demonstrated that the continuation of the trial will cause States, airlines, or any other entity to issue requirements that will in turn cause Petitioner to be vaccinated against Petitioner's will"* even though the Petitioner specifically pointed out such a probability. However, as soon as the FDA approved the Pfizer vaccine, the New York Times published a report titled "Employers Can Require Workers to Get Covid-19 Vaccine - The federal Equal Employment Opportunity Commission said employees could be barred from the workplace if they refused the vaccine." [44] The Health News reported that the International Air Transport Association is in the final stages of developing a digital COVID-19 vaccine passport for travelers. [45] The Los Angeles Unified School District Superintendent already stated on record that students in the Los Angeles school district will have to be vaccinated before returning to the classroom. [46] Therefore, the FDA is cognizant that vaccine requirements are being rolled out by schools, industries, and International Air Transport operators to force large segments of the citizens, some against their will, to be injected with a potentially harmful vaccine, whose efficacy was evaluated using a preliminary test method whose false positive and negative rates are unknown, and which lacks FDA approval. It has been reported that a survey of the staff in a Chicago's west side community hospital right before the vaccine came out showed that 40 percent of health care workers actually said they would not get the vaccine when it was their turn to take the vaccine [47] provided they had a choice.

11. Under **III. C. 1. b. Petitioner Has Not Demonstrated Sound Public Policy Grounds Supporting the Stay** of the Letter, the FDA states the following:

Petitioner does not make any argument about sound public policy, but Petitioner does assert that the public interest weighs in favor of the requested relief "because improving the inaccurate determination of primary endpoints (i) will comport with the best scientific practices, (ii) increase public confidence in the efficacy of a product likely to be mandated or intended for widespread use, and (iii) not doing so will have the opposite result and create uncertainties regarding the efficacy of and need for the COVID-19 vaccines." PSA at 3.

We do not agree that Petitioner has demonstrated sound public policy grounds supporting a stay. Petitioner seeks a stay of a Phase 3 clinical trial. Although the mechanism by which FDA may "stay" a clinical trial is to issue a clinical hold, Petitioner has not identified any basis under 21 CFR § 312.42 or section 505(i)(3) of the FD&C Act for any clinical trial that would justify a clinical hold.

We conclude that a stay of a clinical trial is warranted only when a basis has been demonstrated for a clinical hold in accordance with 21 CFR 312.42 and section 505(i)(3) of the FD&C Act. Because Petitioner has not identified any such basis, we disagree that Petitioner has demonstrated sound public policy grounds supporting the requested stay. We note that if FDA becomes aware of circumstances justifying clinical holds, FDA will order clinical holds in accordance with 21 CFR § 312.42 and section 505(i)(3) of the FD&C Act.

We also note that we disagree with the Petitioner's justification for the request that PCR clinical diagnoses of COVID-19 be followed with Sanger-based sequencing (see discussion above). It would not be sound public policy to require testing protocols that lack scientific merit. Requiring scientifically-unjustified protocols would add unnecessary costs to the clinical trial process, which could disincentivize important medical research."

To support these assertions, the FDA repeatedly declared: "[57...] *we do not agree with Petitioner that it is problematic for clinical trials to use PCR testing of study participants. We also do not agree with Petitioner that the proposed solution—following PCR diagnoses with Sanger-based sequencing—is necessary. Therefore, we do not agree with Petitioner's assertion that there is harm to begin with."*

The FDA refused to accept the fact that the PCR testing system used for study of the vaccine trial participants in a population where the average daily rate of very mild COVID-19 cases, if confirmed, per 100,000 was as low as 3.7, is problematic. Numerous publications, including many from the WHO and the FDA as cited in this rebuttal, had already pointed out by December 2020 the potential false-positives and false-negatives generated by the EUA RT-qPCR tests permitted for preliminary detection of SARS-CoV-2 in clinical specimens. The Petitioner objects to the use of preliminary test results as the pivotal criteria to qualify COVID-19 cases as the endpoints for vaccine efficacy evaluation. The fact that the FDA rejected Sanger-based sequencing for confirmation of PCR test results as "testing protocols that lack scientific merit" shows that the agency does not understand that all NAATs are designed to determine the nucleotide sequence of the target DNA and that PCR is just a tool used to prepare the template for nucleotide sequence analysis. Alternatively, the FDA might have deliberately made such declaration to achieve a non-science-based agenda. In either case, the agency's recommendation for approval of the Pfizer vaccine without diligent review of the raw data should be rescinded to protect the interests of the public.

12. Under **III. C. 1. c. Delay Would Be Outweighed by Public Health or Other Public Interests** of the Letter, the FDA states the following:

> *"We conclude that staying clinical trials without justification would not be in the public health or public interest, and Petitioner has not set forth any justification under our regulations for staying trials that are under FDA IND. The interests of public health would not be served if a stay interfered with the conduct of clinical trials without justification."*
>
> The FDA claimed that the stay would have delayed the conduct of clinical trials. This is untrue. In the Petition to Stay, the Petitioner stated "***Based on an MPR report published on November 8, 2020, there are only 180 confirmed cases of COVID-19 in this clinical trial series that have been analyzed to support the vaccine efficacy evaluation. If the Sponsor (BioNTech/Pfizer) is unable to perform confirmatory Sanger sequencing tests on these 180 RNA extract residual samples, the Petitioner hereby offers to re-test them immediately with Sanger sequencing and submit the laboratory data to support FDA's evaluation. Therefore, there is no excuse for the Sponsor to refuse using the gold standard Sanger sequencing technology for endpoint validation***."
>
> The FDA knew or should have known that it would take 2 days to five days at the most for Pfizer to re-test the residues of the maximum 180 PCR-positive samples by Sanger sequencing to obtain irrefutable evidence to support its claimed 95% vaccination efficacy in prevention of COVID-19. It is disingenuous for the agency to claim that a delay of the vaccine approval for 2-5 days required for re-testing 180 sample residues to gain public confidence in the vaccine's efficacy would have outweighed Public Health or Other Public Interests. The re-testing is especially crucial to support the claimed 95% vaccine efficacy in view of an alternative calculation showing that the vaccine efficacy is actually between 19% and 29%, [48] a figure much lower than the 95% as claimed.

13. Under **III. C. 2. Neither the Public Interest nor the Interest of Justice Support Granting a Discretionary Stay of Action** of the Letter, the FDA states the following:

> *It is in the public interest and the interest of justice to ensure that clinical trials for COVID-19 vaccines continue to determine whether there are vaccines that meet all relevant regulatory requirements. Stays (or clinical holds) may only be justified when there is a basis to do so under 21 CFR § 312.42 and section 505(i)(3) of the FD&C Act. It is not in the public interest or the interest of justice to stay clinical trials in response to a Petition that fails to demonstrate any justification under 21 CFR § 312.42 and section 505(i)(3) of the FD&C Act for a hold. Furthermore, if we required unnecessary steps in the testing to confirm COVID-19 diagnoses, the public interest would not be served because clinical trials should not be required to include*

protocols that lack scientific merit. Requiring scientifically-unjustified protocols would add unnecessary costs to the clinical trial process, which could disincentivize important medical research.

The FDA's claim that an extra 2-5 days re-testing to confirm the 180 preliminary positive test results would be against public interest or the interest of justice, and could disincentivize important medical research, is disingenuous. It is the FDA's agreement to accept data generated using an obviously flawed procedure that lacks scientific merit. Using added unnecessary costs to the clinical trial process as an excuse for the FDA's inaction is absurd because the Petitioner has offered the Sanger retesting for all 180 positive samples free of charge, and will submit the data to the FDA for evaluation.

14. Under **IV. Conclusion** of the Letter, the FDA states the following:

"FDA has considered Petitioner's requests as they relate to the "study design for the Phase III trial[] of BNT162b (NCT04368728)" and COVID-19 vaccine clinical trials. For the reasons given in this letter, FDA denies the requests in the CP and also denies the requests in the PSA. Therefore, we deny the Petitions in their entirety."

This is an arbitrary and capricious conclusion.

According to the Fact Sheet published by the FDA, each dose of the Pfizer-BioNTech COVID-19 Vaccine contains 30 mcg of a nucleoside-modified messenger RNA (modRNA) encoding the viral spike (S) glycoprotein of SARS-CoV-2. The modRNA in the Pfizer-BioNTech COVID-19 Vaccine is formulated in lipid particles, which enable delivery of the RNA into host cells to allow expression of the SARS-CoV-2 S antigen. The vaccine elicits an immune response to the S antigen, which protects against COVID-19. [49]

According to the Pfizer Phase 3 trial protocol, "8.1. Efficacy and/or Immunogenicity Assessments," the definition of confirmed COVID-19 is:

presence of at least 1 of the following symptoms and SARS-CoV-2 NAAT-positive during, or within 4 days before or after, the symptomatic period, either at the central laboratory or at a local testing facility (using an acceptable test):

- *Fever;*
- *New or increased cough;*
- *New or increased shortness of breath;*
- *Chills;*
- *New or increased muscle pain;*
- *New loss of taste or smell;*
- *Sore throat;*
- *Diarrhea;*
- *Vomiting.*

Since the mild clinical symptoms listed above are non-specific for COVID-19, the pivotal criterion for qualifying COVID-19 in the clinical trials is "SARS-CoV-2 NAAT-positive". How to define "SARS-CoV-2 NAAT-positive" is the fundamental question in Pfizer vaccine efficacy evaluation. The vaccine must be proven truly effective in protecting against COVID-19 before it is used in the general population because the long-term safety of such a new vaccine in various segments of the population is unknown.

The Pfizer vaccine is the first of any prophylactic mRNA vaccines scheduled to be injected into healthy humans without a safety-and-efficacy track record. In principle, the synthetic mRNA encoding the spike protein (S protein) of SARS-CoV-2 is packaged as stable nanoparticles consisting of ionizable cationic lipids, natural phospholipids, cholesterol and polyethylene glycol (PEG). The purpose is to direct the human cells to produce a virus protein as an antigen. If successful, the virus protein produced by the host cells will serve as a subunit virus antigen to stimulate immune responses in the host. However, subunit and synthetic peptide/protein antigens by themselves are relatively weak immunogens and require the assistance of specially designed adjuvants to generate a robust and persistent immune response. The stable nanoparticles composed of ssRNA coated with phospholipids have self-adjuvanting properties after being transfected into the cytoplasm by endocytosis. After entering the endosomal/lysosomal compartments of the cell, these adjuvants can activate certain toll-like receptors to initiate a series of innate immune responses, which are required to boost antibody production. And PEG can extend the half-life of these nanoparticles in the host after injection. Since ssRNAs are potent TLR 7/8 agonists and phospholipids are potent TLR 4 agonists, they will activate a series of toll-like receptors, which will lead to strong and long-lasting adaptive immune responses through tumor necrosis factor-α (TNF-α), interferon-γ (IFN- γ) and other proinflammatory cytokines that are secreted by activated immune cells.[41] However, TNF-α and IFN-γ may cause serious adverse effects in certain genetically and physically predisposed individuals.

Since the 1980s, TNF-α, especially in combination with IL-1β, has been known to cause myocardial depression in animals and humans with potentially fatal outcomes. [50-52] Some of the sudden unexpected deaths after injection of the Pfizer vaccine have been reported in the news media. For example, a formerly healthy 41-year-old female healthcare worker died unexpectedly 48 hours after injection of the Pfizer coronavirus vaccine. [53] On January 27, 2021, a local news radio reported that a 60-years old X-ray technician at South Coast Global Medical Center in Santa Ana, California died after receiving 2nd Pfizer vaccine. The deceased apparently died of uncontrollable hypotension and renal failure. [54] These and other similar unexpected deaths after injection of the Pfizer vaccine cannot always be explained away by declaring “lack of evidence linking vaccination to death”. In fact, these unexpected heart failures may be caused by a sudden discharge of TNF-α by macrophages with activated toll-like receptors while these activated cells were clustering in the myocardium. Another vaccine, Gardasil, which is also known to contain viral nucleic acids according to an FDA announcement [55], has been reported to be associated with unexpected deaths among healthy vaccinees after receiving Gardasil injections. At autopsy, these unexpected death cases may show no cause of death. The anatomical findings in the myocardium may range from totally normal to extensive inflammatory cell infiltration. [56, 57]

IFN-γ is known to play important roles in the pathogenesis of autoimmune neuroinflammation, which under certain conditions may lead to multiple sclerosis. [58]

Overproduction of TNF-α in women may lead to obstetric complications, such as recurrent pregnancy loss, early and severe pre-eclampsia, and recurrent implantation failure syndrome. [59]

Therefore, there is scientific evidence in the public domain to suggest that potential health risks may be associated with injection of mRNA vaccines into the human body. The FDA is responsible to ensure that the benefits of the newly introduced mRNA vaccine indeed outweigh its potential risks. The primary benefit for the American citizens who consent to be injected with this potentially harmful vaccine is to reduce the risk of getting SARS-CoV-2 infection, or the risk of becoming a case of COVID-19. Since the Phase 3 clinical trial for the efficacy of the Pfizer mRNA vaccine was primarily based on surveys of the participants developing a mild nonspecific symptom associated with a presumptive positive RT-qPCR result for SARS-CoV-2 RNA in a nasopharyngeal swab

specimen, the presumptive positive RT-qPCR test results were the pivotal criteria to qualify COVID-19 cases as the endpoints for vaccine efficacy evaluation. As none of the RT-qPCR tests used to obtain the presumptive positive results for endpoint determination in the Pfizer vaccine clinical trials have been compared and verified with an FDA-approved test, it is reasonable to request that the FDA demand the vaccine manufacturer to re-test the residues of the presumptive positive samples with a Sanger sequencing method to prove that every one of the 170 to 180 presumptive positive samples in fact contains a segment of SARS-CoV-2 genome. This would be the minimum requirement to gain trust of the American citizens who may be forced to take this vaccine in order to return to normal life. The FDA has not presented a science-based reason to support its arbitrary and capricious denial of the requests in the Citizen's Petition and in the Petition for Stay of Action.

By denying the requests in the Citizen's Petition and in the Petition for Stay of Action, the FDA has deprived the American citizens of their basic rights to informed consent, which must be made based on reliable truthful clinical trial data presented to the FDA for evaluation.

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Petitioner

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