PETENT: **01. Dezember 2020**
Dr. med. Wolfgang Wodarg
Wiesenweg 40
24646 Wächter
Deutschland

CO-PETENT:
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AN:
Europäische Arzneimittel-Agentur (EMA)
Ausschuss für Humanarzneimittel (CHMP)
COVID-19 Pandemie-Arbeitsgruppe der EMA (COVID-ETF)
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!! EILT !!

PETITION BETREFFEND /ANTRAG AUF ADMINISTRATIVE(R)/REGULATORISCHE(R) MASSNAHMEN BETREFFEND BESTÄTIGUNG DER WIRKSAMKEITSENDPUNKTE UND VERWENDUNG DER DATEN IM ZUSAMMENHANG MIT DER/DEN FOLGENDEN KLINISCHEN STUDIE(N):

PHASE III - EUDRACT-NUMMER: 2020-002641-42

NUMMER DES SPONSORPROTOKOLLS: C4591001

SPONSOR:
BIONTECH SE (SOCIETAS EUROPAEA), AN DER GOLDGRUBE 12, 55131 MAINZ, DEUTSCHLAND

UND ALLE ANDEREN KLINISCHEN STUDIEN ZU IMPFSTOFFKANDIDATEN, WELCHE DIE ÜBERTRAGUNG DES VIRUS VOM IMPFSTOFFEMPFÄNGER AUF ANDERE STOPPEN UND/ODER COVID-19 VERHINDERN ODER DIE SYMPTOME VON COVID-19 LINDERN SOLLEN, UND IN WELCHEN PCR-ERGEBNISSE DER PRIMÄRE BEWEIS EINER INFEKTION MIT SARS-COV-2 SIND

ADMINISTRATIVE/REGULATORISCHE AUSSETZUNG

Diese Petition/dieser Antrag auf Aussetzung wird vom Unterzeichner ("**Petent**" oder "**Hauptpetent**") eingereicht, um die EMA zu ersuchen, a) die klinische(n) Phase-III-Studie(n) von BNT162b (EudraCT-Nummer 2020-002641-42) in der EU (gegenwärtiges Protokoll-Land:

Deutschland) so lange auszusetzen, bis das Studiendesign so geändert wurde, dass es den im Abschnitt "Erwünschte Maßnahmen" (**B.**) dargelegten Anforderungen entspricht; und b) alle anderen klinischen Studien mit Impfstoffkandidaten, die darauf abzielen, die Übertragung des Virus vom Impfstoffempfänger auf andere zu stoppen und/oder die Symptome von COVID-19, für die PCR-Ergebnisse der primäre Nachweis einer Infektion sind, zu verhindern oder zu lindern, aussetzt.

Aufgrund der zwingenden Notwendigkeit, die Sicherheit und Wirksamkeit jedes von der EMA (und/oder dem deutschen Paul-Ehrlich-Institut) genehmigten COVID-19-Impfstoffs zu gewährleisten und dem Petenten die Möglichkeit zu geben, angemessene gerichtliche Nothilfe zu beantragen, falls seine Petition ablehnt wird, **bittet der Petent respektvoll darum, dass die EMA unverzüglich auf die sofortige Petition reagiert.**

A. BETROFFENE ENTSCHEIDUNGEN

I. Genehmigung des Studiendesigns und/oder Entscheidung, das Studiendesign für die Phase III-Studie mit BNT162 nicht in Frage zu stellen (EudraCT-Nummer 2020-002641-42).

II. Genehmigung des Studiendesigns und/oder Entscheidung, das Studiendesign aller anderen klinischen Versuche mit Impfstoffkandidaten, die darauf abzielen, die Übertragung des Virus vom Impfstoffempfänger auf andere zu stoppen und/oder die Symptome von COVID-19 zu verhindern oder zu lindern, für die die PCR-Ergebnisse der primäre Nachweis einer Infektion sind, nicht in Frage zu stellen.

B. ERWÜNSCHTE MASSNAHMEN

I. Aussetzen der Phase III-Studie mit BNT162 im Protokollland Deutschland und ggf. in anderen EU-Protokollländern, bis das Studiendesign wie folgt geändert wurde:

Bevor eine Notfallzulassung/bedingte Zulassung und/oder uneingeschränkte Zulassung für den Pfizer/BioNTech-Impfstoff erteilt wird, sollte der Infektionsstatus betreffend aller "Endpunkte" oder COVID-19-Fälle, die zur Bestimmung der Impfstoffwirksamkeit in den Phase-III- oder II/III-Studien verwendet werden, durch eine geeignete Sanger-Sequenzierung (wie in Abschnitt **B. III.** unten beschrieben) bestätigt werden, da a) in einigen Studien hohe Zyklenwerte (Ct-Werte) verwendet werden; und b) bestimmte RT-qPCR-Tests, welche identisch mit dem gelegentlich als "Drosten-Test" bezeichneten Test sind oder diesem nachgebildet wurden, Designfehler aufweisen.

II. Die klinischen Studien zu allen Impfstoffkandidaten, für welche die PCR-Ergebnisse der primäre Nachweis einer Infektion sind und die darauf ausgelegt sind, die Übertragung des Virus vom Impfstoffempfänger auf andere zu stoppen und/oder die Symptome von COVID-19, zu verhindern oder abzuschwächen, werden so lange ausgesetzt bis das Studiendesign wie folgt geändert wird:

Bevor eine Notfallzulassung/bedingte Zulassung und/oder uneingeschränkte Zulassung für einen Impfstoff erteilt wird, der die Übertragung des Virus vom Impfstoffempfänger auf andere stoppen und/oder die Symptome von COVID-19 verhindern oder lindern soll, sollte der Infektionsstatus betreffend aller "Endpunkte" oder COVID-19-Fälle, die zur Bestimmung der Impfstoffwirksamkeit herangezogen werden, durch eine geeignete Sanger-Sequenzierung (wie in Abschnitt **B. III.** unten beschrieben) bestätigt werden, da a) in einigen Studien hohe Zyklenwerte (Ct-Werte) verwendet werden; und b) bestimmte RT-qPCR-Tests, welche identisch mit dem gelegentlich als "Drosten-Test" bezeichneten Test sind oder diesem nachgebildet wurden, Designfehler aufweisen.

III. Hohe Zyklenzahlen (Ct-Werte) bei RT-qPCR-Tests führen anerkanntermaßen zu falsch-positiven Ergebnissen. Zudem hat eine Gruppe von Wissenschaftlern und Forschern vor kurzem eine Rücknahme des Papiers gefordert, das den so genannten "Drosten-Test" beschreibt (manchmal auch als "Corman-Drosten-Protokoll" bezeichnet - ein spezifischer RT-qPCR-Test, der von Corman, Victor M., Drosten, Christian und anderen in "Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR" Euro Surveillance 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045) beschrieben wird.

Alle RT-qPCR-positiven Testergebnisse, die verwendet werden, um Patienten/Probanden in den Studien als "COVID-19-Fälle" zu kategorisieren und um die Endpunkte der Studie zu qualifizieren, sollten durch die Sanger-Sequenzierung verifiziert werden, um zu bestätigen, dass die getesteten Proben tatsächlich eine einzigartige genomische SARS-CoV-2-RNA enthalten. Übereinstimmend mit den Anforderungen der FDA und der EMA für eine bestätigte Diagnose des humanen Papillomavirus (HPV) mittels PCR muss das durch Sequenzierung ausgegebene Elektropherogramm mindestens 100 zusammenhängende Basen zeigen, die mit der Referenzsequenz mit einem Erwartungswert (E-Wert) <10-30 für die spezifische SARS-CoV-2-Gensequenz übereinstimmen, basierend auf einer BLAST-Suche in der GenBank-Datenbank (alias NCBI-Nukleotid-Datenbank).

C. BEGRÜNDUNG

I. Wie hier im Einzelnen dargelegt, (i) würden der Petent und viele EU-Bürger/in der EU ansässige Personen ohne die beantragte Aussetzung einen irreparablen Schaden erleiden; (ii) ist der Antrag nicht missbräuchlich und wird in gutem Glauben gestellt, (iii) manifestiert sich mit dem Antrag eine vernünftige öffentliche Ordnung und (iv) spricht das öffentliche Interesse für die Gewährung einer Aussetzung.

II. Der Petent hält die derzeitigen Studiendesigns für die Phase II/III-Studien von BNT162b ("die Pfizer/BioNTech-Studie") für unzureichend um die Wirksamkeit genau beurteilen zu können. Der Petent hält auch die Designs der klinischen Versuche mit Impfstoffkandidaten, für die die PCR-Ergebnisse der primäre Beweis für eine Infektion sind, und die darauf abzielen, die Übertragung des Virus vom Impfstoffempfänger auf andere zu stoppen und/oder die Symptome von COVID-19 zu verhindern oder zu lindern, für unzureichend, um die Wirksamkeit genau beurteilen zu können.

III. Dem Petenten und der Öffentlichkeit wird ein irreparabler Schaden zugefügt, wenn die hierin geforderten Maßnahmen nicht gewährt werden, denn sobald die EMA (und andere zuständige Stellen in den verschiedenen EU-Mitgliedstaaten) die betreffenden COVID-19-Impfstoffe genehmigt hat, werden sowohl die Regierungen der EU-Mitgliedstaaten als auch die Arbeitgeber in der EU diese Impfstoffe höchstwahrscheinlich für eine breite Anwendung empfehlen. Wenn die Zuordnung von Fällen und Nicht-Fällen (in Bezug auf COVID-19) im Verlauf der Studien nicht korrekt ist, sind die Impfstoffe nicht ordnungsgemäß getestet worden. Wenn die Impfstoffe nicht ordnungsgemäß getestet werden, dürften wichtige politische Entscheidungen über ihren Einsatz auf irreführenden Beweisen beruhen. Die medizinischen und wirtschaftlichen Folgen für die EU-Mitgliedsstaaten und ihre Einwohner/Bürger könnten kaum höher sein.

IV. Wenn die Impfstoffe ohne eine angemessene und genaue Überprüfung der Wirksamkeit zugelassen werden, dann basiert eine mögliche Genehmigung oder ein Vorschreiben dieser Impfstoffe wahrscheinlich auf ungenauen Beweisen bezüglich des Impfstoffs, nämlich dass er die Übertragung des Virus vom Impfstoffempfänger auf andere stoppt und/oder dass er die COVID-19-Krankheit und Todesfälle verringert. Das Pfizer/BioNTech-Studienprotokoll und andere Studienprotokolle sind derzeit nicht darauf ausgelegt, festzustellen, ob eines dieser Ziele erreicht werden kann; und selbst wenn dies der Fall wäre, könnte keines der beiden Ziele zuverlässig erreicht werden, wenn Fälle (in Bezug auf COVID-19) nicht zuverlässig identifiziert werden können,

V. Das öffentliche Interesse an der beantragten Aussetzung wiegt auch deswegen schwer, da eine Verbesserung der genauen Bestimmung der primären Endpunkte (i) mit den besten wissenschaftlichen Praktiken übereinstimmt, (ii) das Vertrauen der Öffentlichkeit in die Wirksamkeit eines Produkts/Impfstoffes, der/welches wahrscheinlich vorgeschrieben oder für eine weit verbreitete Anwendung bestimmt sein wird, erhöht und (iii) bei Nichtaussetzung das gegenteilige Ergebnis eintreten wird und Unsicherheiten hinsichtlich der Wirksamkeit und Notwendigkeit der COVID-19-Impfstoffe schafft.

VI. Der Petent übernimmt hiermit die Gründe, Fakten, Argumente und Meinungen, die in der "PETITION FOR ADMINISTRATIVE ACTION REGARDING CONFIRMATION OF EFFICACY END POINTS OF THE PHASE III CLINICAL TRIALS OF COVID-19 VACCINES" (PETITION FÜR VERWALTUNGSAKTION IN BEZUG AUF DIE BESTÄTIGUNG DER EFFICACY END POINTS OF THE PHASE III CLINICAL TRIALS OF COVID-19 VACCINES“ dargelegt sind, die am 25. November 2020 von Dr. Sin Hang Lee bei der FDA elektronisch eingereicht wurde (**Anlage A** - Docket No. FDA-2020-P-2225). Die beigefügte **Anlage A** ist Bestandteil dieser Petition und ist so zu verstehen, als ob sie im Hauptteil dieser Petition enthalten wäre.

VII. Der Petent greift hiermit auch die Gründe, Fakten, Argumente und Meinungen auf, die im externen Peer-Review des "Drosten-Tests" (**Anlage B**) dargelegt wurden. Designfehler bestimmter RT-qPCR-Tests, die mit dem manchmal als "Drosten-Test" bezeichneten Test identisch sind oder diesem nachempfunden wurden, können zu falsch-positiven Ergebnissen in klinischen Studien führen, die so konzipiert sind, dass die PCR-Ergebnisse den primären Beweis für eine Infektion darstellen. Der dieser Petition beigefügte **Anhang B** ist Bestandteil dieser Petition und ist so zu verstehen, als ob sie im Hauptteil dieser Petition enthalten wäre.

VIII. Damit ein Impfstoff wirkt, muss unser Immunsystem zur Produktion eines neutralisierenden Antikörpers angeregt werden (im Gegensatz zu einem nicht neutralisierenden Antikörper). Ein neutralisierender Antikörper ist ein Antikörper, der eine bestimmte Region ("Epitop") des Virus erkennen und sich an diese Region ("Epitop") binden kann und der in der Folge dazu führt, dass das Virus entweder nicht in ihre Zellen eindringt oder sich nicht in ihren Zellen vermehrt. Ein nicht neutralisierender Antikörper ist ein Antikörper, der sich an das Virus binden kann, aber aus irgendeinem Grund die Infektiosität des Virus nicht neutralisieren kann. Wenn bei einigen Viren eine Person einen nicht neutralisierenden Antikörper gegen das Virus in sich trägt, kann eine nachfolgende Infektion durch das Virus dazu führen, dass diese Person aufgrund des Vorhandenseins des nicht neutralisierenden Antikörpers eine schwerwiegendere Reaktion auf das Virus haben. Dies gilt nicht für alle, sondern nur für bestimmte Viren. Dies wird als antikörperabhängige Verstärkung (Antibody Dependent Enhancement, ADE) bezeichnet und ist ein häufiges Problem bei dem Dengue-Virus, Ebola-Virus, HIV, RSV und Viren aus der Familie der Coronaviren. Tatsächlich ist dieses ADE-Problem einer der Hauptgründe für das Scheitern vieler früherer Impfstoffversuche für andere Coronaviren. Größere Sicherheitsprobleme wurden in Tierversuchen beobachtet. Wenn ADE bei einer Person auftritt, kann ihre Reaktion auf das Virus schlimmer sein als die Reaktion, die auftreten würde, wenn sie von vornherein keine Antikörper entwickelt hätte. Dies kann zu einer hyperinflammatorischen Reaktion, einem Zytokinsturm und einer allgemeinen Dysregulierung des Immunsystems führen, die es dem Virus ermöglicht, unsere Lungen und andere Organe unseres Körpers stärker zu schädigen. Darüber hinaus sind neue Zelltypen in unserem gesamten Körper aufgrund des zusätzlichen viralen Eintrittsweges nun anfällig für eine Virusinfektion. Es gibt viele Studien, die zeigen, dass ADE ein anhaltendes Problem bei Coronaviren im Allgemeinen und insbesondere bei SARS-verwandten Viren ist. ADE hat sich bei Coronavirus-Impfstoffen als ernsthafte Herausforderung erwiesen, und dies ist der Hauptgrund dafür, dass viele dieser Impfstoffe in frühen In-vitro- oder Tierversuchen versagt haben. Beispielsweise zeigten Rhesusaffen, die mit dem Spike-Protein des SARS-CoV-Virus geimpft wurden, schwere akute Lungenschäden, als sie mit SARS-CoV infiziert wurden, während hingegen Affen, die nicht geimpft waren, keine Lungenschäden aufwiesen. In ähnlicher Weise zeigten Mäuse, die mit einem von vier verschiedenen SARS-CoV-Impfstoffen geimpft waren, histopathologische Veränderungen in der Lunge mit eosinophiler Infiltration, nachdem sie mit dem SARS-CoV-Virus infiziert worden waren.

IX. Es gibt einige Probleme mit den klinischen Prüfprotokollen, welche von Dr. Peter Doshi im British Medical Journal dargelegt wurden. Dr. Doshi konzentriert sich dabei auf die beiden größten Probleme. Erstens ist keine der führenden Impfstoffkandidaten-Studien darauf ausgelegt, zu testen, ob der Impfstoff schwere COVID-19-Symptome, definiert als: Krankenhauseinweisungen, Intensivstation oder Tod, reduzieren kann. Und zweitens sollen die Studien nicht testen, ob der Impfstoff die Übertragung unterbrechen kann (https://www.bmj.com/content/bmj/371/bmj.m4037.full.pdf). Wenn keine dieser beiden Bedingungen erfüllt ist, verhält sich der Impfstoff im Wesentlichen wie ein therapeutisches Medikament, mit der Ausnahme, dass ein Impfstoff prophylaktisch auch von völlig Gesunden eingenommen werden würde und höchstwahrscheinlich ein höheres Verletzungs-/Schädigungsrisiko birgt als ein therapeutisches Medikament. Wenn dies zuträfe, dann wären therapeutische Medikamente jedem COVID-Impfstoff überlegen.

X. Im mRNA-Impfstoffkandidaten von Pfizer/BioNTech findet sich Polyethylenglykol (PEG) in der die mRNA umgebende Beschichtung aus Fettlipid-Nanopartikeln. Siebzig Prozent der Menschen stellen Antikörper gegen PEG her und die meisten wissen dieses nicht, was eine

besorgniserregende Situation schafft, in der viele von ihnen allergische, möglicherweise tödliche Reaktionen auf einen PEG-haltigen Impfstoff haben könnten. PEG-Antikörper können auch die Wirksamkeit des Impfstoffs verringern. Pfizer/BioNTech bringt auch einen von einem wirbellosen Meerestier stammenden Inhaltsstoff, mNeonGreen, in seinen Impfstoff ein. Der Inhaltsstoff hat biolumineszierende Eigenschaften, was ihn für medizinische Bildgebungszwecke attraktiv macht, aber es ist dabei unklar, warum ein injizierter Impfstoff diese Eigenschaft aufweisen muss. mNeonGreen hat eine unbekannte Antigenität.

XI. Von mehreren Impfstoffkandidaten wird erwartet, dass sie die Bildung humoraler Antikörper gegen Spike-Proteine von SARS-CoV-2 induzieren. Syncytin-1 (siehe Gallaher, B. , "Response to nCoV2019 Against Background of Endogenous Retroviruses" - http://virological.org/t/response-to-ncov2019-against-backdrop-of-endogenous-retroviruses/396), welches von humanen endogenen Retroviren (HERV) abstammt und für die Entwicklung einer Plazenta bei Säugetieren und Menschen verantwortlich ist und damit eine wesentliche Voraussetzung für eine erfolgreiche Schwangerschaft darstellt, findet sich in homologer Form auch in den Spike-Proteinen von SARS-Viren. Es gibt keine Hinweise darauf, ob Antikörper gegen Spike-Proteine von SARS-Viren auch wie Anti-Syncytin-1-Antikörper wirken würden. Sollte dies jedoch der Fall sein, würde dies auch die Bildung einer Plazenta verhindern, was dazu führen würde, dass geimpfte Frauen im Grunde genommen unfruchtbar werden. Meines Wissens hat Pfizer/BioNTech noch keine Muster des schriftlichen Materials, welches den Patienten/Probanden zur Verfügung gestellt wurde, freigegeben, so dass unklar ist, ob und welche Informationen über (potenzielle) fertilitäts- oder schwangerschaftsspezifischer Risiken infolge der Antikörperbildung enthalten sind.

Gemäß Abschnitt 10.4.2 des Studienprotokolls von Pfizer/BioNTech ist eine Frau mit gebärfähigem Potenzial (WOCBP) teilnahmeberechtigt, wenn sie nicht schwanger ist oder stillt und während des Interventionszeitraums (mindestens 28 Tage nach der letzten Dosis der Studienintervention) eine akzeptable Verhütungsmethode, wie im Studienprotokoll beschrieben, anwendet.

Das bedeutet, dass es relativ lange dauern könnte, bis eine nennenswerte Anzahl von Fällen von Unfruchtbarkeit nach der Impfung beobachtet werden könnte.

XII. Es scheint, dass Pfizer/BioNTech noch keine Muster des schriftlichen Materials, welches den Patienten zur Verfügung gestellt wurde, herausgegeben haben, so dass unklar ist, welche Anweisungen/Informationen Patienten/Probanden bezüglich ADE- und PEG-bezogener Themen und (potenzieller) fertilitäts- oder schwangerschaftsspezifischer Risiken gegeben wurden, wenn überhaupt.

D. AUSSETZUNG DRINGEND GEBOTEN

I. Wenn die EMA den/die betreffenden COVID-19-Impfstoff(e) erst einmal genehmigt hat, werden sowohl die Regierungen der EU-Mitgliedstaaten als auch die Arbeitgeber in der EU den Impfstoff höchstwahrscheinlich für eine breite Anwendung empfehlen, und ohne dass die EMA *jetzt* ordnungsgemäße Sicherheitsprüfungen der Impfstoffe gewährleistet, werden der Petent und andere nicht die Möglichkeit haben, *später* aufgrund mangelhafter klinischer Studien Einwände gegen den Erhalt des Impfstoffs zu erheben.

II. Wenn die Impfstoffe ohne eine angemessene Wirksamkeitsprüfung und ohne

Verbesserung der genauen Bestimmung der primären Endpunkte zugelassen werden, dann basiert eine mögliche Akzeptanz oder ein Vorschreiben dieser Impfstoffe wahrscheinlich auf ungenauen Überzeugungen und Daten über die Impfstoffe, nämlich dass sie die Übertragung des Virus vom Impfstoffempfänger auf andere stoppen oder stoppen könnten und/oder dass sie die schwere COVID-19-Erkrankung und Todesfälle verringern. Die hier gegenständlichen Studienprotokolle sind derzeit nicht geeignet, um festzustellen, ob eines dieser Ziele erreicht werden kann.

III. Auch ist diese Petition nicht missbräuchlich und wird in gutem Glauben eingereicht, da sie darauf abzielt, die wissenschaftliche Integrität und Zuverlässigkeit der Studien zu den COVID-19-Impfstoffen zu erhöhen.

IV. Schließlich spricht auch das öffentliche Interesse sehr für die beantragte Aussetzung, da eine Verbesserung der genauen Bestimmung der primären Endpunkte (i) mit den besten wissenschaftlichen Praktiken übereinstimmt, (ii) das Vertrauen der Öffentlichkeit in die Wirksamkeit eines Impfstoffs, der voraussichtlich vorgeschrieben oder für eine breite Anwendung dringend empfohlen wird, erhöht und (iii) bei Nichtbeachtung das gegenteilige Ergebnis hat, da sie Unsicherheiten hinsichtlich der Wirksamkeit und des Bedarfs an den COVID-19-Impfstoffen schafft.

V. Der Petent drängt daher respektvoll darauf, dass dieser Petition unverzüglich stattgegeben wird.

Hochachtungsvoll eingereicht im eigenen Namen und namens des Co-Petenten Dr. Michael Yeadon:

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Dr. med. Wolfgang Wodarg

Anlage A
Anlage B

Anlage A

VIA ELECTRONIC FILING

November 25, 2020

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

UNITED STATES DEPARTMENT OF HEALTH AND HUMAN SERVICES AND THE FOOD AND DRUG ADMINISTRATION

PETITION FOR ADMINISTRATIVE ACTION REGARDING CONFIRMATION OF EFFICACY END POINTS OF THE PHASE III CLINICAL TRIALS OF COVID-19 VACCINES	**:**	**Docket No. FDA-2020-P-2225**

ADMINISTRATIVE STAY OF ACTION

This petition for a stay of action is submitted on behalf of Dr. Sin Hang Lee ("**Petitioner**") pursuant to 21 C.F.R. § 10.35 and related and relevant provisions of the Federal Food, Drug, and Cosmetic Act or the Public Health Service Act to request the Commissioner of Food and Drugs (the "**Commissioner**") stay the Phase III trials of BNT162b (NCT04368728) to conform with the requests in the "Action Requested" section below.

Because of the compelling need to ensure the safety and efficacy of any COVID-19 vaccine licensed by the FDA, and to allow Petitioner the opportunity to seek emergency judicial relief should the Commissioner deny its Petition, **Petitioner respectfully requests that FDA act on the instant Petition by December 11, 2020.**

A. DECISION INVOLVED

1. Approval of trial design for Phase III trial of BNT162 (NCT04368728)[1]

[1] NCT04368728 available at https://www.clinicaltrials.gov/ct2/show/NCT04368728 (last visited November 3, 2020).

B. ACTION REQUESTED

2. Stay the Phase III trial of BNT162 (NCT04368728) until its study design is amended to provide that:

> Before an EUA or unrestricted license is issued for the Pfizer vaccine, or for other vaccines for which PCR results are the primary evidence of infection, all "endpoints" or COVID-19 cases used to determine vaccine efficacy in the Phase 3 or 2/3 trials should have their infection status confirmed by Sanger sequencing, given the high cycle thresholds used in some trials. High cycle thresholds, or Ct values, in RT-qPCR test results have been widely acknowledged to lead to false positives.[2]
>
> All RT-qPCR-positive test results used to categorize patient as "COVID-19 cases" and used to qualify the trial's endpoints should be verified by Sanger sequencing to confirm that the tested samples in fact contain a unique SARS-CoV-2 genomic RNA. Congruent with FDA requirements for a confirmed diagnosis of human papillomavirus (HPV) using PCR, the sequencing electropherogram must show a minimum of 100 contiguous bases matching the reference sequence with an Expected Value (E Value) $<10^{-30}$ for the specific SARS-CoV-2 gene sequence based on a BLAST search of the GenBank database (aka NCBI Nucleotide database).

C. STATEMENT OF GROUNDS

3. As detailed herein, (i) without the requested stay, the Petitioner will suffer irreparable harm, (ii) the request is not frivolous and is being pursued in good faith, (iii) the request demonstrates sound public policy, and (iv) the public interest favors granting a stay. [3]

4. The current study designs for the Phase II/III trials of BNT162b ("**the Pfizer Vaccine**") are inadequate to accurately assess efficacy.

5. Petitioner and the public will suffer irreparable harm if the actions requested herein are not granted, because once the FDA licenses this COVID-19 vaccine, both governments and employers may make this product mandatory (in general, or for airline or international travel) or may recommend it for widespread use. If the assignment of cases and non-cases during the course of the trial is not accurate, the vaccine will not have been properly tested. If the vaccine is not

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[2] *See* New York Times. Your Coronavirus Test Is Positive. Maybe It Shouldn't Be. By Apoorva Mandavilli. Published Aug. 29, 2020 and updated Sept. 17, 2020, available at https://www.nytimes.com/2020/08/29/health/coronavirus-testing.html.

[3] The Petitioner hereby incorporates by reference as if fully set forth herein the Statement of Grounds from its Citizen's Petition, dated November 23, 2020, available at, https://beta.regulations.gov/document/FDA-2020-P-2225 (last visited November 25, 2020).

properly tested, important public policy decisions regarding its use will be based on misleading evidence. The medical and economic consequences to the nation could hardly be higher.

6. The New York State Bar Association has already issued a report on COVID-19 recommending that, "a vaccine subject to scientific evidence of safety and efficacy be made widely available, and widely encouraged, and if the public health authorities conclude necessary, required…"[4] Thus, it is reasonable to suspect that COVID-19 vaccines, including the Pfizer vaccine, could become mandatory. Without the FDA assuring proper efficacy trials of the vaccine now, the Petitioner and the public may not have the opportunity to object to receiving the vaccine, which was approved based on currently deficient and unreliable clinical trial data.

7. Furthermore, if the vaccine is approved without an appropriate and accurate review of efficacy, then any potential acceptance or mandate of these vaccines is likely to be based on inaccurate evidence regarding the vaccine, namely that it will stop transmission of the virus from the vaccine recipient to others and/or that it will reduce severe COVID-19 disease and deaths. The Pfizer trial protocol is currently not designed to determine whether either of those objectives can be met; and even if it was, if cases cannot be reliably identified, neither objective could be reliably met.

8. The public interest also weighs strongly in favor of the requested relief because improving the accurate 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.

7. According to the trial protocol, "8.1. Efficacy and/or Immunogenicity Assessments," the trial's primary endpoint is prevention of symptomatic disease in vaccine recipients. In order to evaluate that endpoint, the trial will track recorded COVID-19 disease. 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.

[4] https://nysba.org/app/uploads/2020/06/2b-REV-6-12-20-FINAL-HOD-RESOLUTIONS-1-through-4.pdf.

8. As a result, if a participant has a positive reverse transcription-quantitative polymerase chain reaction ("**RT-qPCR**") test along with a cough or sore throat, that participant would be considered as a "confirmed COVID-19 case" and would be counted as an endpoint. Once a trial reaches a certain number of "endpoints", the trial is closer to seeking FDA approval or licensure by demonstrating that the vaccine is "effective" (in that the vaccine group had lower incidence of endpoints than the control group).

9. This effectively means that the efficacy of the vaccine will be determined based on only symptoms of non-specific disease in conjunction with a PCR positive laboratory test.

10. According to the trial protocol, "8.1 Efficacy and/or Immunogenicity Assessments," efficacy will be assessed throughout a participant's involvement in the study through surveillance for potential cases of COVID-19. If, at any time, a participant develops acute respiratory illness (see Section 8.13), for the purposes of the study he or she will be considered to potentially have COVID-19 illness. In this circumstance, the participant should contact the site, an in-person or telehealth visit should occur, and assessments should be conducted as specified in the SoA. The assessments will include a nasal (midturbinate) swab, which will be tested at a central laboratory using a reverse transcription–polymerase chain reaction (RT-PCR) test (Cepheid; FDA approved under EUA), or other equivalent nucleic acid amplification–based test (ie, NAAT), to detect SARS-CoV-2. In addition, clinical information and results from local standard-of-care tests (as detailed in Section 8.13) will be assessed. The central laboratory NAAT result will be used for the case definition, unless no result is available from the central laboratory, in which case a local NAAT result may be used if it was obtained using 1 of the following assays:

- Cepheid Xpert Xpress SARS-CoV-2
- Roche cobas SARS-CoV-2 real-time RT-PCR test (EUA200009/A001)
- Abbott Molecular/RealTime SARS-CoV-2 assay (EUA200023/A001)

11. These test kits referred to in the trial protocol, namely the Cepheid Xpert Xpress SARS-CoV-2, the Roche cobas SARS-CoV-2 real-time RT-PCR test (EUA200009/A001), and the Abbott Molecular/RealTime SARS-CoV-2 assay (EUA200023/A001), are very unreliable tools when they are used to determine whether the nasal swab sample collected from a symptomatic participant contains SARS-CoV-2 or not. These real-time RT-PCR or RT-quantitative PCR tests should be referred to as rRT-PCR or RT-qPCR tests to be distinguished from conventional RT-PCR. The very short RT-qPCR product (amplicon) cannot be analyzed by automated Sanger sequencing as the products of conventional PCR can. And DNA sequencing for validation of the PCR products is needed to correctly determine if the presumptive RT-qPCR-positive SARS-CoV-2 test result is a true positive or a false positive. The reasoning is further outlined as follows:

a. Nowadays DNA sequencing of the PCR amplicon of the genomic nucleic acid of the pathogen is a universally accepted technology for detection and for confirmation of infectious agents, especially pathogenic viruses, in clinical specimens. On January 10,

2020, the first SARS-CoV-2 genome sequence was released online. On the same day, a group of American scientists, most from the CDC, immediately designed 2 complementary panels of primers to amplify the virus genome for sequencing. The PCR amplicons averaged 550 bp in size in their research.[5]

b. The World Health Organization (WHO) guidance titled "WHO Laboratory testing for coronavirus disease (COVID-19) in suspected human cases-Interim guidance dated 19 March 2020" advised "Routine confirmation of cases of COVID-19 is based on detection of unique sequences of virus RNA by NAAT such as real-time reverse transcription-polymerase chain reaction (rRT-PCR) with confirmation by nucleic acid sequencing when necessary."[6]

c. The FDA also recognizes the inherent inaccuracy of the RT-qPCR tests. In its letter issued on February 4, 2020 authorizing emergency use of the CDC 2019-Novel Coronavirus (2019-nCoV, renamed as SARS-CoV-2) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel, the FDA specifically stated that the test panel is "for the ***presumptive*** qualitative detection of nucleic acid from the 2019-nCoV (sic) in upper and lower respiratory specimens."[7]

d. In addition to false-negative results, these RT-qPCR test kits under EUA also generate false-positive test results. For example, 77 positive SARS-CoV-2 test results on a group of football players all turned out to be false positives on repeat tests.[8]

e. The FDA has officially alerted clinical laboratory staff and health care providers of an increased risk of false-positive results with some of these commercial test kits permitted to be used under EUA.[9]

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[5] Paden CR, Tao Y, Queen K, Zhang J, Li Y, Uehara A, Tong S. Rapid, Sensitive, Full-Genome Sequencing of Severe Acute Respiratory Syndrome Coronavirus 2. Emerg Infect Dis. 2020 Oct;26(10):2401-2405. doi: 10.3201/eid2610.201800. Epub 2020 Jul 1. PMID: 32610037; PMCID: PMC7510745.

[6] WHO Laboratory testing for coronavirus disease (COVID-19) in suspected human cases-Interim guidance 19 March 2020. Available from: https://www.who.int/publications/i/item/10665-331501.

[7] FDA letter dated February 4, 2020 authorizing emergency use of the CDC 2019-Novel Coronavirus (2019-nCoV, renamed as SARS-CoV-2) Real-Time Reverse Transcriptase (RT)-PCR Diagnostic Panel. See Open letter from FDA to Robert R. Redfield, MD, Director, Centers for Disease Control and Prevention. March 15, 2020. https://www.fda.gov/media/134919/download.

[8] Kevin Patra. Around the NFL- All 77 false-positive COVID-19 tests come back negative upon reruns. Aug 24, 2020. Available from: https://www.nfl.com/news/all-77-false-positive-covid-19-tests-come-back-negative-upon-reruns.

[9] FDA. False Positive Results with BD SARS-CoV-2 Reagents for the BD Max System - Letter to Clinical Laboratory Staff and Health Care Providers. Available from: https://www.fda.gov/medical-devices/letters-health-care-providers/false-positive-results-bd-sars-cov-2-reagents-bd-max-system-letter-clinical-laboratory-staff-and Accessed November 2, 2020; *see also* FDA. Risk of Inaccurate Results with Thermo Fisher Scientific TaqPath COVID-19 Combo Kit - Letter to Clinical Laboratory Staff and Health Care Providers. Available from: https://www.fda.gov/medical-devices/letters-health-care-providers/risk-inaccurate-results-thermo-fisher-scientific-taqpath-covid-19-combo-kit-letter-clinical?utm_campaign=2020-08-17%20Risk%20of%20Inaccurate%20Results%20with%20Thermo%20Fisher%20Scientific%20TaqPath&utm_medium=email&utm_source=Eloqua.

f. To resolve the problems caused by these inherently inaccurate tests, the FDA's position is that false results can be investigated using an additional EUA RT-qPCR assay, and/or Sanger sequencing.[10] Since an additional EUA RT-qPCR test result may also generate a false result, Sanger sequencing is the *de facto* gold standard for confirmation of presumptive qualitative detection of nucleic acid from the SARS-CoV-2 and for excluding false-positive cases.

g. 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 on both strands of the PCR amplicon (bi-directional sequencing) that contains a minimum of 100 contiguous bases is acceptable as valid diagnostics for HPV infection provided the sequence matches the reference or consensus sequence, e.g. with an Expected Value (E Value) $<10^{-30}$ for the specific HPV DNA target based on a BLAST search of the GenBank (NCBI Nucleotide) database.[11] Following this FDA guidance, and showing the feasibility of implementing the FDA guidance for accurate diagnosis of COVID-19, a protocol using the nested PCR cDNA amplicon of a 398-base highly conserved SARS-CoV-2 N gene segment as the template for Sanger sequencing was developed for confirmatory detection of SARS-CoV-2 in clinical samples.[12]

h. DNA sequencing verification is necessary for confirmation of the presumptive SARS-CoV-2-positive cases in the Pfizer vaccine's Phase II/III clinical trial because, according to its Protocol, the specimens collected from the symptomatic trial subjects were sent to a central laboratory using a reverse transcription–polymerase chain reaction (RT-PCR) test (Cepheid; FDA approved under EUA), or other equivalent nucleic acid amplification–based test (i.e., NAAT), to detect SARS-CoV-2.

   In order to raise the detection sensitivity, the mean Ct value of the Cepheid system is set as high as 42.9 for the N2 target, and as high as 44.9 for the E target, as shown in Table 4 of Instructions for Users (Cepheid 302-3562, Rev. E September 2020).[13]

[10] FDA. Molecular Diagnostic Template for Laboratories. Policy for Coronavirus Disease-2019 Tests During the Public Health Emergency (Revised) Available from: https://www.fda.gov/media/135659/download .

[11] FDA. Establishing the Performance Characteristics of In Vitro Diagnostic Devices for the Detection or Detection and Differentiation of Human Papillomaviruses. Available from: https://www.fda.gov/media/92930/download.

[12] Lee SH. Testing for SARS-CoV-2 in cellular components by routine nested RT-PCR followed by DNA sequencing. International Journal of Geriatrics and Rehabilitation. 2020; 2:69-96. Available from: http://www.int-soc-clin-geriat.com/info/wpcontent/uploads/2020/03/Dr.-Lees-paper-on-testing-for-SARS-CoV-2.pdf.

[13] Cepheid. GeneXpert. Instructions for Users. XPRSARS-COV2-10. 302-3562, Rev. E September 2020 https://www.cepheid.com/Package%20Insert%20Files/Xpress-SARS-CoV-2/Xpert%20Xpress%20SARS-CoV-2%20Assay%20ENGLISH%20Package%20Insert%20302-3562-GX%20Rev.%20E.pdf.

Table 4. LoD Determination using USA-WA1/2020 Strain

Strain	Concentration (PFU/mL)	Total Valid Results	Hit Rate (%)	Hit Rate (%)	Mean Ct	Mean Ct
			N2 Target	E Target	N2 Target	E Target
SARS-CoV-2 virus (USA_WA1/2020)	0.0200	20	100	95.0	38.3	36.4
	0.0050	22	95.5	68.2	40.5	39.1
	0.0025	22	90.9	36.4	41.5	39.6
	0.0010	22	50.0	18.2	42.0	42.0
	0.0005	22	45.5	18.2	41.7	41.5
	0.0003	22	18.2	4.5	42.1	44.9
	0.0001	22	9.1	0	42.9	N/A
	0	0	0	0	N/A	N/A

At Ct values between 36.0 and 44.9, many RT-qPCR positive test results are false positives.

i. The results of the 3 RT-qPCR test kits used in the trial protocol are not comparable. A sample identified as negative by the Abbott kit can be classified as positive by the Cepheid kit. According to an FDA survey, the limit of detection by the Cepheid Xpert Xpress SARS-CoV-2 test kit and the limit of detection by Abbott RealTime SARS-CoV-2 assay kit are found to be identical, namely both being at 5400 NAAT Detectable Units/ mL, as shown in the comparative data extracted from an FDA reference panel.[14]

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

However, due to the designation of higher cycle threshold test results as positives, the Cepheid Xpert kits have classified many Abbott kit negative cases as positives in a head-to-head comparative study as shown in the following "Table 2" extracted from a report by Basu et al.[15]

[14] FDA. SARS-CoV-2 Reference Panel Comparative Data. https://www.fda.gov/medical-devices/coronavirus-covid-19-and-medical-devices/sars-cov-2-reference-panel-comparative- data.

[15] *See* bioRxiv preprint doi: https://doi.org/10.1101/2020.05.11.089896; Basu A, Zinger T, Inglima K, Woo KM, Atie O, Yurasits L, See B, Aguero-Rosenfeld ME. Performance of Abbott ID Now COVID-19 Rapid Nucleic Acid Amplification Test Using Nasopharyngeal Swabs Transported in Viral Transport Media and Dry Nasal Swabs in a New York City Academic Institution. J Clin Microbiol. 2020 Jul 23;58(8):e01136-20. doi: 10.1128/JCM.01136-20. PMID: 32471894; PMCID: PMC7383552.

Table 2. Results of sequential nasopharyngeal specimens submitted in VTM from the Emergency Department tested on both Abbot ID NOW and Cepheid GeneXpert for SARS CoV-2 RNA

Sample ID	Abbott IDNOW Result*	Cepheid Result	N2 Ct	E Ct
1	Negative	Positive	43.1	0.0
2	Negative	Positive	40.7	37.0
3	Positive	Positive	32.4	29.0
4	Positive	Positive	32.3	30.3
5	Positive	Positive	18.2	16.2
6	Positive	Positive	31.6	28.5
7	Positive	Positive	35.1	31.3
8	Negative	Positive	44.1	0.0
9	Negative	Positive	44.3	0.0
10	Positive	Positive	29.7	27.1
11	Positive	Positive	27.6	26.2
12	Positive	Positive	19.7	17.5
13	Positive	Positive	18.6	16.2
14	Negative	Positive	36.3	33.3
15	Positive	Positive	23.7	26.5

j. One of the Cepheid Xpert kit users has put out an alert, stating "The instruments are presently set by the manufacturer to interpret a single target positive with very poor amplification efficiency (high Cycle Threshold [Ct] and/or atypical curve) as 'DETECTED.' None of these to date have confirmed positive when tested on other systems using similar targets, and may be a false positive due to background noise."[16]

k. Another group of users also found that some tested samples classified as positives by the Cepheid test kits cannot be confirmed with other test kits. These authors published a report, stating: "We found that the sensitivity of the Xpert Xpress SARS-CoV-2 assay was 100% (20 of 20) and the specificity was 80% (16 of 20). When looking at the cycle threshold (Ct) values from the GeneXpert assay we observed that specimens with no amplification of the *E* gene (ie, Ct=0) and Ct values for the *N2* gene greater than 40 cycles were considered as positives, whereas they were negative using the other RT-PCR system (Da An Gene)."[17]

[16] Diagnostic Laboratory Services Inc. Technical Alert. Cepheid GeneXpert and BD Max Instruments may be Reporting False Positives. https://dlslab.com/documents/bulletins/2020/tech-memo-sars-cov-2-pcr-possible-false-positive-6-19-2020.pdf.

[17] Rakotosamimanana N, Randrianirina F, Randremanana R, Raherison MS, Rasolofo V, Solofomalala GD, Spiegel A, Heraud JM. GeneXpert for the diagnosis of COVID-19 in LMICs. Lancet Glob Health. 2020 Oct 19:S2214-109X(20)30428-9. doi: 10.1016/S2214-109X(20)30428-9. Epub ahead of print. PMID: 33091372; PMCID: PMC7572106.

12. DNA sequencing verification of the RT-qPCR positive test results is absolutely necessary in this placebo-controlled randomized clinical trial because *de facto* unblinding has occurred among the participants. According to the trial protocol Section 8.13. COVID-19 Surveillance (All Participants), "If a participant experiences any of the following (irrespective of perceived etiology or clinical significance), he or she is instructed to contact the site immediately and, if confirmed, participate in an in-person or telehealth visit as soon as possible." This contact would trigger an automatic NAAT test by a Cepheid RT-qPCR assay at the central laboratory or at a local laboratory by any similar acceptable methods.

At the time of enrollment, the participants were informed that each of them would be injected with a vaccine to protect against COVID-19 infection or a saline placebo without disclosing which one of the two was injected into the participant. However, all participants were also informed that the vaccine may cause the following reactions:

- Fever ≥39.0°C (≥102.1°F).
- Redness or swelling at the injection site measuring greater than 10 cm (>20 measuring device units).
- Severe pain at the injection site.
- Any severe systemic event.

It is commonly known to the general public and especially to the informed clinical trial participants that intramuscular injection of a very small amount of sterile normal saline will not cause fever, local redness and swelling, and severe pain, or systemic reactions. The participants receiving placebo would intuitively or reasonably know that they were not injected with a vaccine and were not protected against COVID-19 disease due to the lack of any vaccine reaction after the injection. As a result, more participants receiving placebo than those receiving vaccine would report to the "site" manager when they developed any minor symptoms, such as a sore throat or a new cough for the fear of coming down with COVID-19. The site manager must investigate the symptoms reported, including ordering a RT-qPCR test by Cepheid assay to be performed at the Central Laboratory according to Protocol. The more severe cases might be tested locally by Abbott kits or Roche kits because they might have to be tested in the hospital after admission, and because many hospitals are aware of the high false positive rates generated by the Cepheid kits. The results generated by these test kits are not comparable since the Cepheid test kits using a very high Ct value up to 44.9 for "detection of SARS-CoV-2 genomic RNA" **tend to generate many more false positives than the other test kits**. A higher number of false-positive test results in the participants receiving placebo will artificially raise the efficacy of the vaccine, unless the RT-qPCR test results are verified by nucleotide sequencing to eliminate all false-positive test results.

13. 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.[18] If the Sponsor (BioNTech/Pfizer) is unable to perform confirmatory Sanger sequencing tests on these 180 RNA extract residual samples, the Petitioner hereby offers

[18] Diana Ernst, RPh. Final Analysis Reveals COVID-19 Vaccine Candidate BNT162b2 95% Effective. MPR Report. November 18, 2020. https://www.empr.com/home/news/drugs-in-the-pipeline/pfizer-biontech-mrna-based-vaccine-bnt162b2-against-covid19-effective/.

to re-test them immediately with Sanger sequencing[19] 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.

14. In summary, based on the scientific data available in the public domain and the FDA guidance, all RT-qPCR test results for detection of SARS-CoV-2 gene sequence must be considered presumptive. The Cepheid test kits for SARS-CoV-2 are known to generate more false-positive test results than other EUA assay kits.

15. The residues of the tested samples that were classified as positive for SARS-CoV-2 by the Cepheid GeneXpert assay, or equivalent as stated in the Pfizer Clinical Trial Protocol, must be re-tested by a Sanger sequencing method to confirm that the presumptive positive samples in fact contain a unique sequence of SARS-CoV-2 genome. Only then can the positive test results from the Cepheid GeneXpert test kits be accepted as an accurate component of the "endpoint." Only then can one nonspecific symptom plus laboratory positivity be accepted as a valid measure of confirmed COVID-19 cases or "endpoints."

Stay Urgently Required

16. Petitioner will suffer irreparable harm because once the FDA licenses this COVID-19 vaccine, states are expected to make this product mandatory, and hence without the FDA assuring proper safety trials of the vaccine *now*, the Petitioner will not have the opportunity to object to receiving the vaccine based on deficient clinical trials *later*.

17. For example, the New York State Bar Association recently passed a resolution recommending that "[s]hould the level of immunity be deemed insufficient by expert medical and scientific consensus to check the spread of COVID-19 and reduce morbidity and mortality, **a mandate and state action should be considered**."[20] Mandating administration of the vaccine, thereby eliminating the right to informed consent, makes acute the need to assure that the safety and efficacy of any COVID-19 vaccine is robustly studied in an adequate clinical trial monitoring for any potential adverse events.

18. Furthermore, if the vaccine is licensed without an appropriate efficacy review and without improving the accurate determination of primary endpoints, then any potential acceptance or mandate of these vaccines are likely to be based on inaccurate beliefs about the vaccine, namely that it will stop transmission of the virus from the vaccine recipient to others or that it will reduce severe COVID-19 disease and deaths. The trial protocols are not currently designed to determine whether either of those objectives can be met.

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[19] Lee SH. Testing for SARS-CoV-2 in cellular components by routine nested RT-PCR followed by DNA sequencing. International Journal of Geriatrics and Rehabilitation. 2020; 2:69-96. Available from: http://www.int-soc-clin-geriat.com/info/wpcontent/uploads/2020/03/Dr.-Lees-paper-on-testing-for-SARS-CoV-2.pdf.

[20] https://nysba.org/app/uploads/2020/11/11.-Health-Law-Section-COVID-19-Report-September-20-2020-with-all-comments.pdf (emphasis added) (last visited November 10, 2020).

19. This request is also not frivolous and is being pursued in good faith as it seeks to increase the scientific integrity and reliability of the trials of the COVID-19 Vaccines.

20. Finally, the public interest also weighs strongly in favor of the requested relief because improving the accurate determination of primary endpoints (i) will comport with the best scientific practices, (ii) increase public confidence in the efficacy of a product expected to be mandated, and (iii) not doing so will have the opposite result in that it will create uncertainties regarding the efficacy of and need for the COVID-19 Vaccines.

21. The Petitioner therefore respectfully urges that this request be granted forthwith.

Respectfully submitted,

Dr. Sin Hang Lee

Review Report by an International Consortium of Scientists in Life Sciences (ICSLS) - Corman-Drosten *et al.*, Eurosurveillance 2020 (Updated: 29.11.2020)

External peer review of the RTPCR test to detect SARS-CoV-2 reveals 10 major scientific flaws at the molecular and methodological level: consequences for false positive results.

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Kevin McKernan[(5)] , Klaus Steger[(6)] , Paul McSheehy[(7)] , Lidiya Angelova[(8)]

Fabio Franchi[(9)], Thomas Binder[(10)], Henrik Ullrich[(11)] , Makoto Ohashi[(12)]

Stefano Scoglio[(13)], Marjolein Doesburg-van Kleffens[(14)], Dorothea Gilbert[(15)]

Rainer Klement[(16)], Ruth Schruefer[(17)], Berber W. Pieksma[(18)], Jan Bonte[(19)]

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ABSTRACT

"In the publication entitled “Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR” (Eurosurveillance 25(8) 2020) the authors present a diagnostic workflow and RT-qPCR protocol for detection and diagnostics of 2019-nCoV (now known as SARS-CoV-2), which they claim to be validated, as well as being a robust diagnostic methodology for use in public-health laboratory settings.

In light of all the consequences resulting from this very publication for societies worldwide, a group of independent researchers performed a point-by-point review of the aforesaid publication in which 1) all components of the presented test design were cross checked, 2) the RT-qPCR protocol-recommendations were assessed with respect to good laboratory practice, and 3) parameters examined against relevant scientific literature covering the field.

The published RT-qPCR protocol for detection and diagnostics of 2019-nCoV and the manuscript suffer from numerous technical and scientific errors, including insufficient primer design, a problematic and insufficient RT-qPCR protocol, and the absence of an accurate test validation. Neither the presented test nor the manuscript itself fulfils the requirements for an acceptable scientific publication. Further, serious conflicts of interest of the authors are not mentioned. Finally, the very short timescale between submission and acceptance of the publication (24 hours) signifies that a systematic peer review process was either not performed here, or of problematic poor quality.

We provide compelling evidence of several scientific inadequacies, errors and flaws. Considering the scientific and methodological blemishes presented here, we are confident that the editorial board of Eurosurveillance has no other choice but to retract the publication."

CONCISE REVIEW REPORT

This paper will show numerous serious flaws in the Corman-Drosten paper, the significance of which has led to worldwide misdiagnosis of infections attributed to SARS-CoV-2 and associated with the disease COVID-19. We are confronted with stringent lockdowns which have destroyed many people's lives and livelihoods, limited access to education and these imposed restrictions by governments around the world are a direct attack on people's basic rights and their personal freedoms, resulting in collateral damage for entire economies on a global scale.

There are ten fatal problems with the Corman-Drosten paper which we will outline and explain in greater detail in the following sections.

The first and major issue is that the novel Coronavirus SARS-CoV-2 (in the publication named 2019-nCoV and in February 2020 named SARS-CoV-2 by an international consortium of virus experts) is based on in silico (theoretical) sequences, supplied by a laboratory in China [1], because at the time neither control material of infectious ("live") or inactivated SARS-CoV-2 nor isolated genomic RNA of the virus was available to the authors. To date no validation has been performed by the authorship based on isolated SARS-CoV-2 viruses or full length RNA thereof. According to Corman et al.:

> *"We aimed to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available."* [1]

The focus here should be placed upon the two stated aims: a) *development* and b) *deployment of a diagnostic test for use in public health laboratory settings*. These aims are not achievable without having any actual virus material available (e.g. for determining the infectious viral load). In any case, only a protocol with maximal accuracy can be the mandatory and primary goal in any scenario-outcome of this magnitude. Critical viral load determination is mandatory information, and it is in Christian Drosten's group responsibility to perform these experiments and provide the crucial data.

Nevertheless these in silico sequences were used to develop a RT-PCR test methodology to identify the aforesaid virus. This model was based on the assumption that the novel virus is very similar to SARS-CoV from 2003 as both are beta-coronaviruses.

The PCR test was therefore designed using the genomic sequence of SARS-CoV as a control material for the Sarbeco component; we know this from our personal email-communication with [2] one of the co-authors of the Corman-Drosten paper. This method to model SARS-CoV-2 was described in the Corman-Drosten paper as follows:

> *"the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, designed in absence of available virus isolates or original*

> *patient specimens. Design and validation were enabled by the close genetic relatedness to the 2003 SARS-CoV, and aided by the use of synthetic nucleic acid technology."*

The Reverse Transcription-Polymerase Chain Reaction (RT-PCR) is an important biomolecular technology to rapidly detect rare RNA fragments, which are known in advance. In the first step, RNA molecules present in the sample are reverse transcribed to yield cDNA. The cDNA is then amplified in the polymerase chain reaction using a specific primer pair and a thermostable DNA polymerase enzyme. The technology is highly sensitive and its detection limit is theoretically 1 molecule of cDNA. The specificity of the PCR is highly influenced by biomolecular design errors.

What is important when designing an RT-PCR Test and the quantitative RT-qPCR test described in the Corman-Drosten publication?

1. The primers and probes:

a) the concentration of primers and probes must be of optimal range (100-200 nM)

b) must be specific to the target-gene you want to amplify

c) must have an optimal percentage of GC content relative to the total nitrogenous bases (minimum 40%, maximum 60%)

d) for virus diagnostics at least 3 primer pairs must detect 3 viral genes (preferably as far apart as possible in the viral genome)

2. The temperature at which all reactions take place:

a) DNA melting temperature (>92°)

b) DNA amplification temperature (TaqPol specific)

c) Tm; the annealing temperature (the temperature at which the primers and probes reach the target binding/detachment, not to exceed 2°C per primer pair). Tm heavily depends on GC content of the primers

3. The number of amplification cycles (less than 35; preferably 25-30 cycles);

In case of virus detection, >35 cycles only detects signals which do not correlate with infectious virus as determined by isolation in cell culture [reviewed in 2]; if someone is tested by PCR as positive when a threshold of 35 cycles or higher is used (as is the case in most laboratories in Europe & the US), the probability that said person is actually infected is less than 3%, the probability that said result is a false positive is 97% [reviewed in 3]

4. Molecular biological validations; amplified PCR products must be validated either by running the products in a gel with a DNA ruler, or by direct DNA sequencing

5. Positive and negative controls should be specified to confirm/refute specific virus detection

6. There should be a Standard Operational Procedure (SOP) available

SOP unequivocally specifies the above parameters, so that all laboratories are able to set up the exact same test conditions. To have a validated universal SOP is essential, because it enables the comparison of data within and between countries.

MINOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

1. In Table 1 of the Corman-Drosten paper, different abbreviations are stated - “nM” is specified, “nm” isn’t. Further in regards to correct nomenclature, nm means “nanometer” therefore nm should read nM here.

2. It is the general consensus to write genetic sequences always in the 5’-3’ direction, including the reverse primers. It is highly unusual to do alignment with reverse complementary writing of the primer sequence as the authors did in figure 2 of the Corman-Drosten paper. Here, in addition, a wobble base is marked as “y” without description of the bases the Y stands for.

3. Two misleading pitfalls in the Corman-Drosten paper are that their Table 1 does not include Tm-values (annealing-temperature values), neither does it show GC-values (number of G and C in the sequences as %-value of total bases).

MAJOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

A) BACKGROUND

The authors introduce the background for their scientific work as: “The ongoing outbreak of the recently emerged novel coronavirus (2019-nCoV) poses a challenge for public health laboratories as virus isolates are unavailable while there is growing evidence that the outbreak is more widespread than initially thought, and international spread through travelers does already occur”.

According to BBC News [4] and Google Statistics [5] there were 6 deaths world-wide on January 21st 2020 - the day when the manuscript was submitted. Why did the authors assume a challenge for public health laboratories while there was no substantial evidence at that time to indicate that the outbreak was more widespread than initially thought?

As an aim the authors declared to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. Further, they acknowledge

that "The present study demonstrates the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks."

B) METHODS AND RESULTS

1. Primer & Probe Design

1a) Erroneous primer concentrations

Reliable and accurate PCR-test protocols are normally designed using between 100 nM and 200 nM per primer [7]. In the Corman-Drosten paper, we observe unusually high and varying primer concentrations for several primers (table 1). For the RdRp_SARSr-F and RdRp_SARSr-R primer pairs, 600 nM and 800 nM are described, respectively. Similarly, for the N_Sarbeco_F and N_Sarbeco_R primer set, they advise 600 nM and 800 nM, respectively [1].

It should be clear that these concentrations are far too high to be optimal for specific amplifications of target genes. **There exists no specified reason to use these extremely high concentrations of primers in this protocol. Rather, these concentrations lead to increased unspecific binding and PCR product amplification.**

Table1: Primers and probes (adapted from Corman-Drosten paper; erroneous primer concentrations are highlighted)

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

1b) Unspecified ("Wobbly") primer and probe sequences

To obtain reproducible and comparable results, it is essential to distinctively define the primer pairs. In the Corman-Drosten paper we observed six unspecified positions, indicated by the letters R, W, M and S (Table 2). The letter W means that at this position there can be

either an A or a T; R signifies there can be either a G or an A; M indicates that the position may either be an A or a C; the letter S indicates there can be either a G or a C on this position. This high number of variants not only is unusual, but it also is highly confusing for laboratories. These six unspecified positions could easily result in the design of several different alternative primer sequences which do not relate to SARS-CoV-2 (2 distinct RdRp_SARSr_F primers + 8 distinct RdRp_SARS_P1 probes + 4 distinct RdRp_SARSr_R). The design variations will inevitably lead to results that are not even SARS CoV-2 related. Therefore, the confusing unspecific description in the Corman-Drosten paper is not suitable as a Standard Operational Protocol. These unspecified positions should have been designed unequivocally.

These wobbly sequences have already created a source of concern in the field and resulted in a Letter to the Editor authored by Pillonel et al. [8] regarding blatant errors in the described sequences. These errors are self-evident in the Corman et al. supplement as well.

Table 2: Primers and probes (adapted from Corman-Drosten paper; unspecified ("Wobbly") nucleotides in the primers are highlighted)

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

The WHO-protocol (Figure 1), which directly derives from the Corman-Drosten paper, concludes that in order to confirm the presence of SARS-CoV-2, two control genes (the E-and the RdRp-genes) must be identified in the assay. It should be noted, that the RdPd-gene has one uncertain position ("wobbly") in the forward-primer (R=G/A), two uncertain positions in the reverse-primer (R=G/A; S=G/C) and it has three uncertain positions in the RdRp-probe (W=A/T; R=G/A; M=A/C). So, two different forward primers,

four different reverse primers, and eight distinct probes can be synthesized for the RdPd-gene. Together, there are 64 possible combinations of primers and probes!

The Corman-Drosten paper further identifies a third gene which, according to the WHO protocol, was not further validated and deemed unnecessary:

"Of note, the N gene assay also performed well but was not subjected to intensive further validation because it was slightly less sensitive."

This was an unfortunate omission as it would be best to use all three gene PCRs as confirmatory assays, and this would have resulted in an almost sufficient virus RNA detection diagnostic tool protocol. Three confirmatory assay-steps would at least minimize-out errors & uncertainties at every fold-step in regards to "Wobbly"-spots. (Nonetheless, the protocol would still fall short of any "good laboratory practice", when factoring in all the other design-errors).

As it stands, the N gene assay is regrettably neither proposed in the WHO-recommendation (Figure 1) as a mandatory and crucial third confirmatory step, nor is it emphasized in the Corman-Drosten paper as important optional reassurance "for a routine workflow" (Table 2).

Consequently, in nearly all test procedures worldwide, merely 2 primer matches were used instead of all three. This oversight renders the entire test-protocol useless with regards to delivering accurate test-results of real significance in an ongoing pandemic.

Figure 1: The N-Gene confirmatory-assay is neither emphasized as necessary third step in the official WHO Drosten-Corman protocol-recommendation below [8] nor is it required as a crucial step for higher test-accuracy in the Eurosurveillance publication.

Background
We used known SARS- and SARS-related coronaviruses (bat viruses from our own studies as well as literature sources) to generate a non-redundant alignment (excerpts shown in Annex). We designed candidate diagnostic RT-PCR assays before release of the first sequence of 2019-nCoV. Upon sequence release, the following assays were selected based on their matching to 2019-nCoV as per inspection of the sequence alignment and initial evaluation (Figures 1 and 2).

All assays can use SARS-CoV genomic RNA as positive control. Synthetic control RNA for 2019-nCoV E gene assay is available via EVAg. Synthetic control for 2019-nCoV RdRp is expected to be available via EVAg from Jan 21st onward.

First line screening assay: E gene assay
Confirmatory assay: RdRp gene assay

1c) Erroneous GC-content (discussed in 2c, together with annealing temperature (Tm))

1d) Detection of viral genes
RT-PCR is not recommended for primary diagnostics of infection. This is why the RT-PCR Test used in clinical routine for detection of COVID-19 is not indicated for COVID-19 diagnosis on a regulatory basis.

"Clinicians need to recognize the enhanced accuracy and speed of the molecular diagnostic techniques for the diagnosis of infections, but also to understand their limitations. Laboratory results should always be interpreted in the context of the clinical presentation of the patient, and appropriate site, quality, and timing of specimen collection are required for reliable test results". [9]

However, it may be used to help the physician's differential diagnosis when he or she has to discriminate between different infections of the lung (Flu, Covid-19 and SARS have very similar symptoms). For a confirmative diagnosis of a specific virus, at least 3 specific primer pairs must be applied to detect 3 virus-specific genes. Preferably, these target genes should be located with the greatest distance possible in the viral genome (opposite ends included).

Although the Corman-Drosten paper describes 3 primers, these primers only cover roughly half of the virus' genome. This is another factor that decreases specificity for detection of intact COVID-19 virus RNA and increases the quote of false positive test results.

Therefore, even if we obtain three positive signals (i.e. the three primer pairs give 3 different amplification products) in a sample, this does not prove the presence of a virus. A better primer design would have terminal primers on both ends of the viral genome. This is

because the whole viral genome would be covered and three positive signals can better discriminate between a complete (and thus potentially infectious) virus and fragmented viral genomes (without infectious potency). In order to infer anything of significance about the infectivity of the virus, the Orf1 gene, which encodes the essential replicase enzyme of SARS-CoV viruses, should have been included as a target (Figure 2). The positioning of the targets in the region of the viral genome that is most heavily and variably transcribed is another weakness of the protocol.

Kim et al. demonstrate a highly variable 3' expression of subgenomic RNA in Sars-CoV-2 [23]. These RNAs are actively monitored as signatures for asymptomatic and non-infectious patients [10]. It is highly questionable to screen a population of asymptomatic people with qPCR primers that have 6 base pairs primer-dimer on the 3 prime end of a primer (Figure 3).

Apparently the WHO recommends these primers. We tested all the wobble derivatives from the Corman-Drosten paper with Thermofisher's primer dimer web tool [11]. The RdRp forward primer has 6bp 3prime homology with Sarbeco E Reverse. At high primer concentrations this is enough to create inaccuracies.

Of note: There is a perfect match of one of the N primers to a clinical pathogen (Pantoea), found in immuno-compromised patients. The reverse primer hits Pantoea as well but not in the same region (Figure 3).

These are severe design errors, since the test cannot discriminate between the whole virus and viral fragments. The test cannot be used as a diagnostic for SARS-viruses.

Figure 2: Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome. ORF: open reading frame; RdRp: RNA-dependent RNA polymerase. Numbers below amplicon are genome positions according to SARS-CoV, NC_004718 [1];

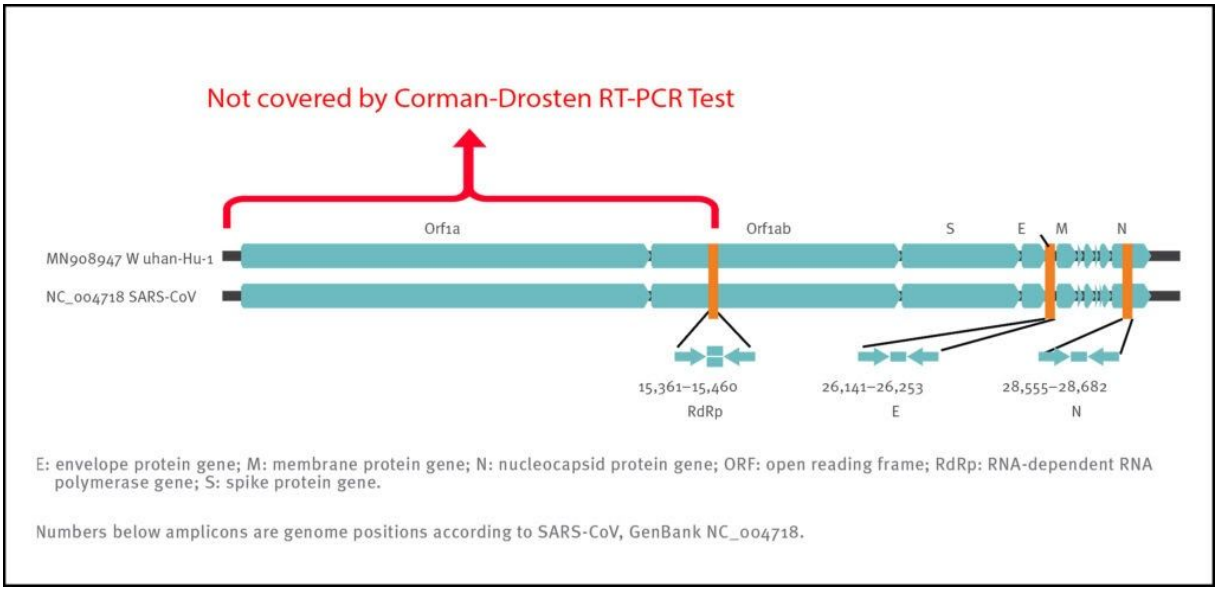
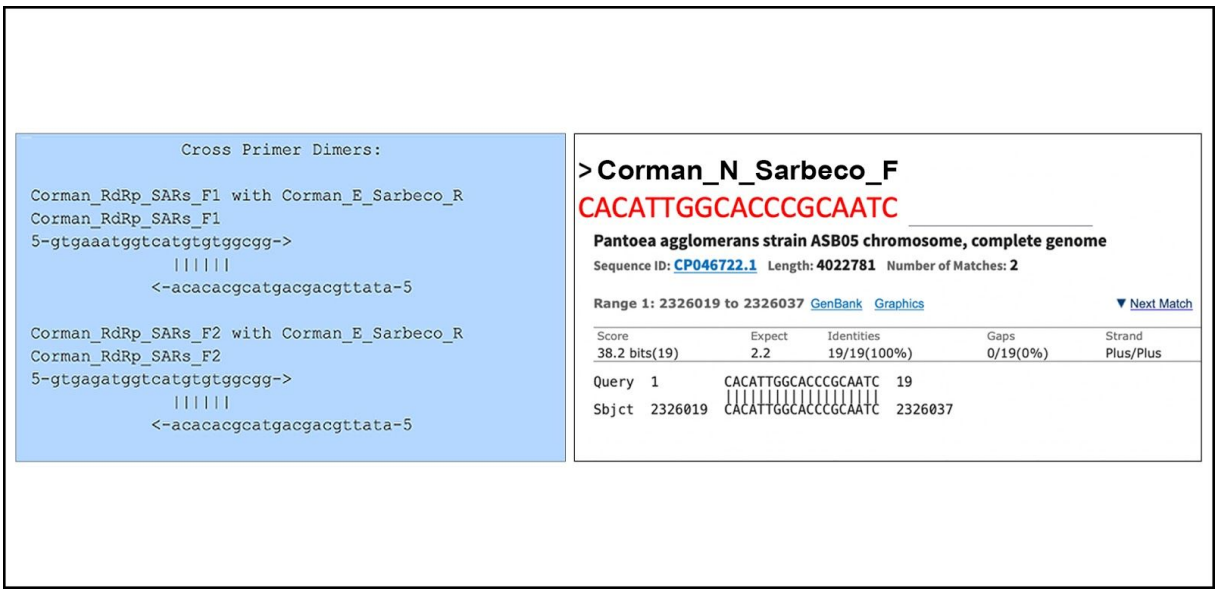


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (Pantoea) found in immuno-compromised patients (right box).



2. Reaction temperature

2a) DNA melting temperature (>92°).
Adequately addressed in the Corman-Drosten paper.

2b) DNA amplification temperature.
Adequately addressed in the Corman-Drosten paper.

2c) Erroneous GC-contents and Tm
The annealing-temperature determines at which temperature the primer attaches/detaches from the target sequence. For an efficient and specific amplification, GC content of primers should meet a minimum of 40% and a maximum of 60% amplification. As indicated in table 3, three of the primers described in the Corman-Drosten paper are not within the normal range for GC-content. Two primers (RdRp_SARSr_F and RdRp_SARSr_R) have unusual and very low GC-values of 28%-31% for all possible variants of wobble bases, whereas primer E_Sarbeco_F has a GC-value of 34.6% (Table 3 and second panel of Table 3).

It should be noted that the GC-content largely determines the binding to its specific target due to its three hydrogen bonds in base pairing. Thus, the lower the GC-content of the primer, the lower its binding-capability to its specific target gene sequence (i.e. the gene to be detected). This means for a target-sequence to be recognized we have to choose a temperature which is as close as possible to the actual annealing-temperature (best practise-value) for the primer not to detach again, while at the same time specifically selecting the target sequence.

If the Tm-value is very low, as observed for all wobbly-variants of the RdRp reverse primers, the primers can bind non-specifically to several targets, decreasing specificity and increasing potential false positive results.

The annealing temperature (Tm) is a crucial factor for the determination of the specificity/accuracy of the qPCR procedure and essential for evaluating the accuracy of qPCR-protocols. Best-practice recommendation: Both primers (forward and reverse) should have an almost similar value, preferably the identical value.

We used the freely available primer design software Primer-BLAST [12, 25] to evaluable the best-practise values for all primers used in the Corman-Drosten paper (Table 3). We attempted to find a Tm-value of 60° C, while similarly seeking the highest possible GC%-value for all primers. A maximal Tm difference of 2° C within primer pairs was considered acceptable. Testing the primer pairs specified in the Corman-Drosten paper, we observed a difference of 10° C with respect to the annealing temperature Tm for primer pair1 (RdRp_SARSr_F and RdRp_SARSr_R). This is a very serious error and makes the protocol useless as a specific diagnostic tool.

Additional testing demonstrated that only the primer pair designed to amplify the N-gene (N_Sarbeco_F and N_Sarbeco_R) reached the adequate standard to operate in a diagnostic test, since it has a sufficient GC-content and the Tm difference between the primers (N_Sarbeco_F and N_Sarbeco_R) is 1.85° C (below the crucial maximum of 2° C difference). Importantly, this is the gene which was neither tested in the virus samples (Table 2) nor emphasized as a confirmatory test. In addition to highly variable melting temperatures and degenerate sequences in these primers, there is another factor impacting specificity of the procedure: the dNTPs (0.4uM) are 2x higher than recommended for a highly specific amplification. There is additional magnesium sulphate added to the reaction as well. This procedure combined with a low annealing temperature can create non-specific amplifications. When additional magnesium is required for qPCR, specificity of the assay should be further scrutinized.

The design errors described here are so severe that it is highly unlikely that specific amplification of SARS-CoV-2 genetic material will occur using the protocol of the Corman-Drosten paper.

Table 3: GC-content of the primers and probes (adapted from Corman-Drosten paper; aberrations from optimized GC-contents are highlighted. Second Panel shows a table-listing of all Primer-BLAST best practices values for all primers and probes used in the Corman-Drosten paper by Prof. Dr. Ulrike Kämmerer & her team.

Normal ranges for GC%: 40 - 60%; normal ranges for TM: 55-65°; Best-practise for qPCR in our case: 60° for both primers (reverse & forward)

GC% 59,09 — TM 63,74°

Difference of almost 10°

GC% 28,00 — TM 53,56°

GC% 34,62 — TM 58,29°

GC% 45,45% — TM 60,93°

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
RdRP gene	RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG	Use 600 nM per reaction
	RdRp_SARSr-P2	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ	Specific for 2019-nCoV, will not detect SARS-CoV. Use 100 nM per reaction and mix with P1
	RdRP_SARSr-P1	FAM-CCAGGTGGWACRTCATCMGGTGATGC-BBQ	Pan Sarbeco-Probe will detect 2019-nCoV, SARS-CoV and bat-SARS-related CoVs. Use 100 nM per reaction and mix with P2
	RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA	Use 800 nM per reaction
E gene	E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Use 400 nm per reaction
	E_Sarbeco_P1	FAM-ACACTAGCCATCCTTACTGCGCTTCG-BBQ	Use 200 nm per reaction
	E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Use 400 nm per reaction
N gene	N_Sarbeco_F	CACATTGGCACCCGCAATC	Use 600 nm per reaction
	N_Sarbeco_P	FAM-ACTTCCTCAAGGAACAACATTGCCA-BBQ	Use 200 nm per reaction
	N_Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Use 800 nm per reaction

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.

[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

		GC	TM	Search in **MN908947** (first full genome from Wuihan, 12.01.2020)						
Primer pairs	**Sequence (5´-3´)**	**Template strand**	**Length**	**Start**	**Stop**	**Tm**	**GC%**	**Self 5' complementarity**	**Self 3' complementarity**	**Product length (bp)**
E_Sarbeco_F	ACAGGTACGTTAATAGTTAATAGCGT	Plus	26	26269	26294	58.29	34.62	8.00	8.00	**113**
E_Sarbeco_R	ATATTGCAGCAGTACGCACACA	Minus	22	26381	26360	60.93	45.45	7.00	1.00	
N-Sarbeco_F	CACATTGGCACCCGCAATC	Plus	19	28706	28724	60.15	57.89	4.00	0.00	**128**
N-Sarbeco_R	GAGGAACGAGAAGAGGCTTG	Minus	20	28833	28814	58.00	55.00	3.00	1.00	
RdRp_SARSr-F	GTGARATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	to be added in next version	
RdRp_SARSr-R	CARATGTTAAASACACTATTAGCATA		25			53.56	28.00	7.00		
If R= G and S= G	GTGAGATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	1.00	
	CAGATGTTAAAGACACTATTAGCATA		26			55.22	30.77	7.00	5.00	not found in the Sequence
If R= G and S= C	GTGAGATGGTCATGTGTGGCGG		22			63.74	59.09	4.00	1.00	
	CAGATGTTAAACACACTATTAGCATA		26			55.68	30.77	7.00	2.00	
If R= A and S= G	GTGAAATGGTCATGTGTGGCGG		22			62.58	54.55	4.00	1.00	
	CAAATGTTAAAGACACTATTAGCATA		26			54.23	26.92	7.00	5.00	
If R= A and S= C	GTGAAATGGTCATGTGTGGCGG		22			62.58	54.55	4.00	1.00	
	CAAATGTTAAACACACTATTAGCATA		26			54.69	26.92	7.00	2.00	
Probes:										
RdRp-SARSr-P2	CAGGTGGAACCTCATCAGGAGATGC		25			64.89	56.00	6.00	5.00	
RdRp-SARSr-P1	CCAGGTGGWACRTCATCMGGTGATGC									
E-Sarbeco-P1	ACACTAGCCATCCTTACTGCGCTTCG		26			66.78	53.85	4.00	2.00	
N-Sarbeco-P	ACTTCCTCAAGGAACAACATTGCCA		25			63.15	44.00	8.00	3.00	

3. The number of amplification cycles

It should be noted that there is no mention anywhere in the Corman-Drosten paper of a test being positive or negative, or indeed what defines a positive or negative result. These types of virological diagnostic tests must be based on a SOP, including a validated and fixed number of PCR cycles (Ct value) after which a sample is deemed positive or negative. The maximum reasonably reliable Ct value is 30 cycles. Above a Ct of 35 cycles, rapidly increasing numbers of false positives must be expected .

PCR data evaluated as positive after a Ct value of 35 cycles are completely unreliable.

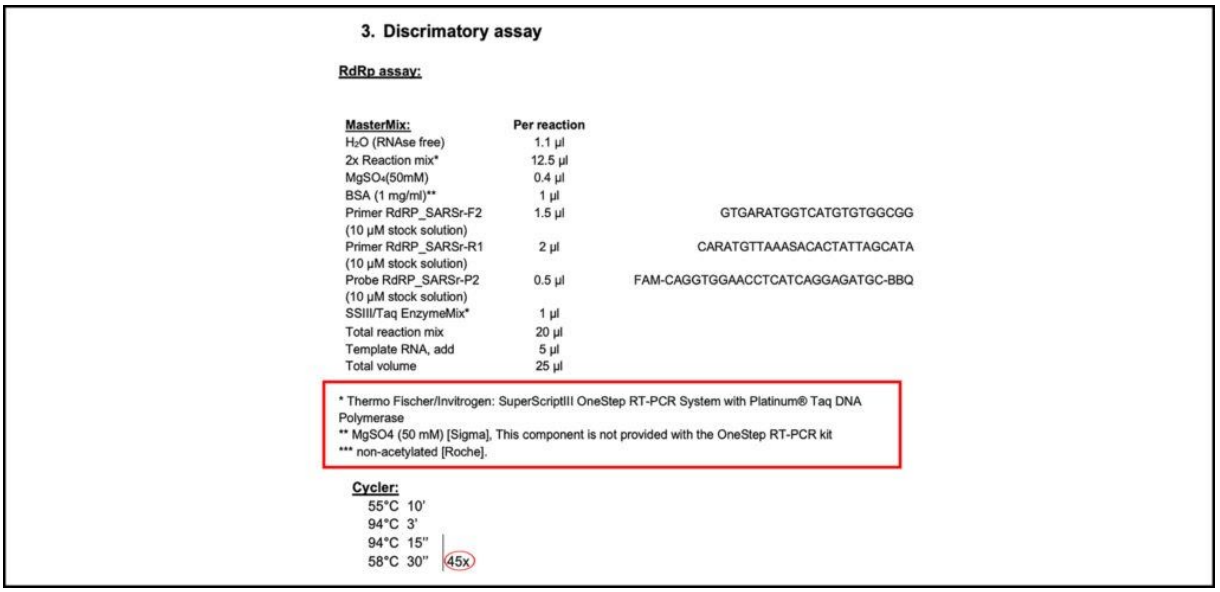
Citing Jaafar et al. 2020 [3]:

"At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive."

In other words, there was no successful virus isolation of SARS-CoV-2 at those high Ct values. Further, scientific studies show that only non-infectious (dead) viruses are detected with Ct values of 35 [22].

Between 30 and 35 there is a grey area, where a positive test cannot be established with certainty. This area should be excluded. Of course, one could perform 45 PCR cycles, as recommended in the Corman-Drosten WHO-protocol (Figure 4), but then you also have to define a reasonable Ct-value (which should not exceed 30). But an analytical result with a Ct value of 45 is scientifically and diagnostically absolutely meaningless (a reasonable Ct-value should not exceed 30). All this should be communicated very clearly. It is a significant mistake that the Corman-Drosten paper does not mention the maximum Ct value at which a sample can be unambiguously considered as a positive or a negative test-result. This important cycle threshold limit is also not specified in any follow-up submissions to date.

Figure 4: RT-PCR Kit recommendation in the official Corman-Drosten WHO-protocol [8]. Only a "Cycler"-value (cycles) is to be found without corresponding and scientifically reasonable Ct (Cutoff-value). This or any other cycles-value is nowhere to be found in the actual Corman-Drosten paper.



3. Discrimatory assay

RdRp assay:

MasterMix:	**Per reaction**	
H_2O (RNAse free)	1.1 µl	
2x Reaction mix*	12.5 µl	
$MgSO_4$(50mM)	0.4 µl	
BSA (1 mg/ml)**	1 µl	
Primer RdRP_SARSr-F2 (10 µM stock solution)	1.5 µl	GTGARATGGTCATGTGTGGCGG
Primer RdRP_SARSr-R1 (10 µM stock solution)	2 µl	CARATGTTAAASACACTATTAGCATA
Probe RdRP_SARSr-P2 (10 µM stock solution)	0.5 µl	FAM-CAGGTGGAACCTCATCAGGAGATGC-BBQ
SSIII/Taq EnzymeMix*	1 µl	
Total reaction mix	20 µl	
Template RNA, add	5 µl	
Total volume	25 µl	

* Thermo Fischer/Invitrogen: SuperScriptIII OneStep RT-PCR System with Platinum® Taq DNA Polymerase
** MgSO4 (50 mM) [Sigma], This component is not provided with the OneStep RT-PCR kit
*** non-acetylated [Roche].

Cycler:
55°C 10'
94°C 3'
94°C 15"
58°C 30" 45x

4. Biomolecular validations

To determine whether the amplified products are indeed SARS-CoV-2 genes, biomolecular validation of amplified PCR products is essential. For a diagnostic test, this validation is an absolute must.

Validation of PCR products should be performed by either running the PCR product in a 1% agarose-EtBr gel together with a size indicator (DNA ruler or DNA ladder) so that the size of the product can be estimated. The size must correspond to the calculated size of the amplification product. But it is even better to sequence the amplification product. The latter will give 100% certainty about the identity of the amplification product. Without molecular validation one can not be sure about the identity of the amplified PCR products. Considering the severe design errors described earlier, the amplified PCR products can be anything.

Also not mentioned in the Corman-Drosten paper is the case of small fragments of qPCR (around 100bp): It could be either 1,5% agarose gel or even an acrylamide gel.

The fact that these PCR products have not been validated at molecular level is another striking error of the protocol, making any test based upon it useless as a specific diagnostic tool to identify the SARS-CoV-2 virus.

5. Positive and negative controls to confirm/refute specific virus detection.

The unconfirmed assumption described in the Corman-Drosten paper is that SARS-CoV-2 is the only virus from the SARS-like beta-coronavirus group that currently causes infections in humans. The sequences on which their PCR method is based are in silico sequences, supplied by a laboratory in China [23], because at the time of development of the PCR test no control material of infectious (“live”) or inactivated SARS-CoV-2 was available to the authors. The PCR test was therefore designed using the sequence of the known SARS-CoV as a control material for the Sarbeco component (Dr. Meijer, co-author Corman-Drosten paper in an email exchange with Dr. Peter Borger) [2].

All individuals testing positive with the RT-PCR test, as described in the Corman-Drosten paper, are assumed to be positive for SARS-CoV-2 infections. There are three severe flaws

in their assumption. First, a positive test for the RNA molecules described in the Corman-Drosten paper cannot be equated to “infection with a virus”. A positive RT-PCR test merely indicates the presence of viral RNA molecules. As demonstrated under point 1d (above), the Corman-Drosten test was not designed to detect the full-length virus, but only a fragment of the virus. We already concluded that this classifies the test as unsuitable as a diagnostic test for SARS-virus infections.

Secondly and of major relevance, the functionality of the published RT-PCR Test was not demonstrated with the use of a positive control (isolated SARS-CoV-2 RNA) which is an essential scientific gold standard.

Third, the Corman-Drosten paper states:

“To show that the assays can detect other bat-associated SARS-related viruses, we used the E gene assay to test six bat-derived faecal samples available from Drexler et al. […] und Muth et al. […]. These virus-positive samples stemmed from European rhinolophid bats. Detection of these phylogenetic outliers within the SARS-related CoV clade suggests that all Asian viruses are likely to be detected. This would, theoretically, ensure broad sensitivity even in case of multiple independent acquisitions of variant viruses from an animal reservoir.”

This statement demonstrates that the E gene used in RT-PCR test, as described in the Corman-Drosten paper, is not specific to SARS-CoV-2.

The E gene primers also detect a broad spectrum of other SARS viruses.

The genome of the coronavirus is the largest of all RNA viruses that infect humans and they all have a very similar molecular structure. Still, SARS-CoV1 and SARS-CoV-2 have two highly specific genetic fingerprints, which set them apart from the other coronaviruses. First, a unique fingerprint-sequence (KTFPPTEPKKDKKKK) is present in the N-protein of SARS-CoV and SARS-CoV-2 [13,14,15]. Second, both SARS-CoV1 and SARS-CoV2 do not contain the HE protein, whereas all other coronaviruses possess this gene [13, 14]. So, in order to specifically detect a SARS-CoV1 and SARS-CoV-2 PCR product the above region in the N gene should have been chosen as the amplification target. A reliable diagnostic test should focus on this specific region in the N gene as a confirmatory test. The PCR for this N gene was not further validated nor recommended as a test gene by the Drosten-Corman paper, because of being “not so sensitive” with the SARS-CoV original probe [1].

Furthermore, the absence of the HE gene in both SARS-CoV1 and SARS-CoV-2 makes this gene the ideal negative control to exclude other coronaviruses. The Corman-Drosten paper does not contain this negative control, nor does it contain any other negative controls. The

PCR test in the Corman-Drosten paper therefore contains neither a unique positive control nor a negative control to exclude the presence of other coronaviruses. This is another major design flaw which classifies the test as unsuitable for diagnosis.

6. Standard Operational Procedure (SOP) is not available

There should be a Standard Operational Procedure (SOP) available, which unequivocally specifies the above parameters, so that all laboratories are able to set up the identical same test conditions. To have a validated universal SOP is essential, because it facilitates data comparison within and between countries. It is very important to specify all primer parameters unequivocally. We note that this has not been done. Further, the Ct value to indicate when a sample should be considered positive or negative is not specified. It is also not specified when a sample is considered infected with SARS-CoV viruses. As shown above, the test cannot discern between virus and virus fragments, so the Ct value indicating positivity is crucially important. This Ct value should have been specified in the Standard Operational Procedure (SOP) and put on-line so that all laboratories carrying out this test have exactly the same boundary conditions. It points to flawed science that such an SOP does not exist. The laboratories are thus free to conduct the test as they consider appropriate, resulting in an enormous amount of variation. Laboratories all over Europe are left with a multitude of questions; which primers to order? which nucleotides to fill in the undefined places? which Tm value to choose? How many PCR cycles to run? At what Ct value is the sample positive? And when is it negative? And how many genes to test? Should all genes be tested, or just the E and RpRd gene as shown in Table 2 of the Corman-Drosten paper? Should the N gene be tested as well? And what is their negative control? What is their positive control?

The protocol as described is unfortunately very vague and erroneous in its design that one can go in dozens of different directions. There does not appear to be any standardization nor an SOP, so it is not clear how this test can be implemented.

7. Consequences of the errors described under 1-5: false positive results.

The RT-PCR test described in the Corman-Drosten paper contains so many molecular biological design errors (see 1-5) that it is not possible to obtain unambiguous results. It is inevitable that this test will generate a tremendous number of so-called “false positives”. The definition of false positives is a negative sample, which initially scores positive, but which is negative after retesting with the same test. False positives are erroneous positive test-results, i.e. negative samples that test positive. And this is indeed what is found in the Corman-Drosten paper. On page 6 of the manuscript PDF the authors demonstrate, that even under well-controlled laboratory conditions, a considerable percentage of false positives is generated with this test:

"In four individual test reactions, weak initial reactivity was seen however they were negative upon retesting with the same assay. These signals were not associated with any particular virus, and for each virus with which initial positive reactivity occurred, there were other samples that contained the same virus at a higher concentration but did not test positive. Given the results from the extensive technical qualification described above, it was concluded that this initial reactivity was not due to chemical instability of real-time PCR probes and most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study." [1]

The first sentence of this excerpt is clear evidence that the PCR test described in the Corman-Drosten paper generates false positives. Even under the well-controlled conditions of the state-of-the-art Charité-laboratory, 4 out of 310 primary-tests are false positives per definition. Four negative samples initially tested positive, then were negative upon retesting. This is the classical example of a false positive. In this case the authors do not identify them as false positives, which is intellectually dishonest.

Another telltale observation in the excerpt above is that the authors explain the false positives away as "handling issues caused by the rapid introduction of new diagnostic tests". Imagine the laboratories that have to introduce the test without all the necessary information normally described in an SOP.

8. The Corman-Drosten paper was not peer-reviewed

Before formal publication in a scholarly journal, scientific and medical articles are traditionally certified by "peer review." In this process, the journal's editors take advice from various experts ("referees") who have assessed the paper and may identify weaknesses in its assumptions, methods, and conclusions. Typically a journal will only publish an article once the editors are satisfied that the authors have addressed referees' concerns and that the data presented supports the conclusions drawn in the paper." This process is as well described for Eurosurveillance [16].

The Corman-Drosten paper was submitted to Eurosurveillance on January 21st 2020 and accepted for publication on January 22nd 2020. On January 23rd 2020 the paper was online. On January 13th 2020 version 1-0 of the protocol was published at the official WHO website [17], updated on January 17th 2020 as document version 2-1 [18], even before the Corman-Drosten paper was published on January 23rd at Eurosurveillance.

Normally, peer review is a time-consuming process since at least two experts from the field have to critically read and comment on the submitted paper. In our opinion, this paper was not peer-reviewed. Twenty-four hours are simply not enough to carry out a thorough peer review. Our conclusion is supported by the fact that a tremendous number of very serious design flaws were found by us, which make the PCR test completely unsuitable as a diagnostic tool to identify the SARS-CoV-2 virus. Any molecular biologist familiar with RT-PCR

design would have easily observed the grave errors present in the Corman-Drosten paper before the actual review process. We asked Eurosurveillance on October 26th 2020 to send us a copy of the peer review report. To date, we have not received this report and in a letter dated November 18th 2020, the ECDC as host for Eurosurveillance declined to provide access without providing substantial scientific reasons for their decision. On the contrary, they write that "disclosure would undermine the purpose of scientific investigations." [24].

9. Authors as the editors

A final point is one of major concern. It turns out that two authors of the Corman-Drosten paper, Christian Drosten and Chantal Reusken, are also members of the editorial board of this journal [19]. Hence there is a severe conflict of interest which strengthens suspicions that the paper was not peer-reviewed. It has the appearance that the rapid publication was possible simply because the authors were also part of the editorial board at Eurosurveillance. This practice is categorized as compromising scientific integrity.

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

1. There exists no specified reason to use these extremely high concentrations of primers in this protocol. The described concentrations lead to increased nonspecific bindings and PCR product amplifications, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

2. Six unspecified wobbly positions will introduce an enormous variability in the real world laboratory implementations of this test; the confusing nonspecific description in the Corman-Drosten paper is not suitable as a Standard Operational Protocol making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

3. The test cannot discriminate between the whole virus and viral fragments. Therefore, the test cannot be used as a diagnostic for intact (infectious) viruses, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus and make inferences about the presence of an infection.

4. A difference of 10° C with respect to the annealing temperature Tm for primer pair1 (RdRp_SARSr_F and RdRp_SARSr_R) also makes the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

5. A severe error is the omission of a Ct value at which a sample is considered positive and negative. This Ct value is also not found in follow-up submissions making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

6. The PCR products have not been validated at the molecular level. This fact makes the protocol useless as a specific diagnostic tool to identify the SARS-CoV-2 virus.

7. The PCR test contains neither a unique positive control to evaluate its specificity for SARS-CoV-2 nor a negative control to exclude the presence of other coronaviruses, making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

8. The test design in the Corman-Drosten paper is so vague and flawed that one can go in dozens of different directions; nothing is standardized and there is no SOP. This highly questions the scientific validity of the test and makes it unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

9. Most likely, the Corman-Drosten paper was not peer-reviewed making the test unsuitable as a specific diagnostic tool to identify the SARS-CoV-2 virus.

10. We find severe conflicts of interest for at least four authors, in addition to the fact that two of the authors of the Corman-Drosten paper (Christian Drosten and Chantal Reusken) are members of the editorial board of Eurosurveillance. A conflict of interest was added on July 29 2020 (Olfert Landt is CEO of TIB-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for TIB-Molbiol), that was not declared in the original version (and still is missing in the PubMed version); TIB-Molbiol is the company which was “the first” to produce PCR kits (Light Mix) based on the protocol published in the Corman-Drosten manuscript, and according to their own words, they distributed these PCR-test kits before the publication was even submitted [20]; further, Victor Corman & Christian Drosten failed to mention their second affiliation: the commercial test laboratory “Labor Berlin”. Both are responsible for the virus diagnostics there [21] and the company operates in the realm of real time PCR-testing.

In light of our re-examination of the test protocol to identify SARS-CoV-2 described in the Corman-Drosten paper we have identified concerning errors and inherent fallacies which render the SARS-CoV-2 PCR test useless.

CONCLUSION

The decision as to which test protocols are published and made widely available lies squarely in the hands of Eurosurveillance. A decision to recognise the errors apparent in the Corman-Drosten paper has the benefit to greatly minimise human cost and suffering going forward.

Is it not in the best interest of Eurosurveillance to retract this paper? Our conclusion is clear. In the face of all the tremendous PCR-protocol design flaws and errors described here, we conclude: There is not much of a choice left in the framework of scientific integrity and responsibility.

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