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http://ag-evolutionsbiologie.net/pdf/2021/Drosten-PCR-Borger-Report.pdf

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23. Januar 2021

Zusammenfassung:
Am 23.Jan. 2020 haben Corman et al. den ersten qPCR-Test auf das neue Virus SARS-CoV-2 veröffentlicht. Am 27. Nov. 2020 erschien im Internet ein Text von Borger et al., in dem besagter Test harsch kritisiert wurde. Der hier vorliegende Text enthält die detaillierte Analyse der Argumente von Borger und Koautoren für alle Interessierten, die es ausführlich nachlesen wollen. Er zeigt, dass Kritik von Borger et al. vollständig unbegründet ist. Die beiden farblich unterschiedenen Versionen (Deutsch / Englisch) sind inhaltlich inhaltsgleich.

Jan. 23th Corman et al. (2020) have published the first qPCR assay for the novel virus SARS-CoV-2. Nov 27th Borger et al. (2020) published a text with harsh attacks on the Corman paper. This text is on the Borger et al. publication. It contains its detailed analysis and refutation - for everyone who wants to have the information detailed and at length. Analysis is given in German and English, in different colours, respectively.

Review report Corman-Drosten et al. Eurosurveillance 2020

CURATED BY AN INTERNATIONAL CONSORTIUM OF SCIENTISTS IN LIFE SCIENCES (ICSLS)

https://cormandrostenreview.com/report/
(27. November 2020; letzter Zugriff 21. Dezember 2020)

This extensive review report has been officially submitted to Eurosurveillance editorial board on 27th November 2020 via their submission-portal, enclosed to this review report is a *retraction* request letter, signed by all the main & co-authors. First and last listed names are the first and second main authors. All names in between are co-authors.

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.

Peter Borger[(1)], Bobby Rajesh Malhotra[(2)], Michael Yeadon[(3)] , Clare Craig[(4)], 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)], Bruno H. Dalle Carbonare[(20)], Kevin P. Corbett[(21)], Ulrike Kämmerer[(22)]

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 w.r.t. good laboratory practice, and 3) parameters examined against relevant scientific literature covering the field.

Das ist weit aus dem Fenster gelehnt. Wenn man solche Kritik ernst meint, sollte man ***Experimente*** machen - der Test ist ja mittlerweile etabliert und frei verfügbar: Man nehme ein paar Dutzend Proben und teste. Warum ist dies seitens der Kritiker nicht geschehen? *Honi soit qui mal y pense*. Außerdem handelt es sich bei den Autoren definitiv ***nicht*** um ein Konsortium von ***(Lebens-/Bio-)Wissenschaftlern*** ("consortium of scientists in life sciences") - mehr dazu am Ende dieses Dokuments.

Bold to say so. If you utter serious critique you should corroborate this with ***hard data***. The pPCR-system developed by Drosten and coworkers is freely available, so it is simple to test it. Why did this not happen? *Honi soit qui mal y pense.* Besides this, the authors clearly are - as claimed by themselves - ***not*** a "consortium of ***scientists in life sciences"*** (details at the end of this document).

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.

Nochmals: Der Test wird und wurde weltweit genutzt, und dann soll niemandem in der Praxis aufgefallen sein, dass er untauglich ist? Und keiner der Fachleute soll bemerkt haben, dass die Ergebnisse dieses Tests nicht mit den Ergebnissen von Antikörper-Suchtests übereinstimmen? Wollen dies die Autoren ernsthaft unterstellen?
Der Test wurde und wird in der Zwischenzeit auch gegenüber anderen qPCR Tests (unabhängige Entwicklungen von Pasteur, CDC, CDC-China, etc.) validiert. Zudem kombinieren verschiedene Dignostika-Hersteller Assays der Charite mit Eigenentwicklungen oder Assays vom CDC in ihren dual-target oder triple-target SARS-CoV-2 Tests - und dies stets mit kongruenten Ergebnissen.

Once again: This test was and is used worldwide. If it truly was faulty, no one has realised this so far? And no one so far has realised, that the results of this test are incompatible with the results of antibody screening? Seriously?
The Corman-Drosten assay meanwhile has been tested and validated against other qPCR tests (independently developed by Pateeuer, CDC, CDC China etc.). Meanwhile, quite a few SARS-CoV-2 double- and triple tests have been developed by different suppliers combining Charité with other qPCR tests. Results always have been compatible.

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.

Das ist ein bemerkenswerter Kommentar. Derlei Anmerkungen haben in wissenschaftlichen Texten eigentlich nichts verloren. Hier kommt ziemlich unverhohlen raus, worum es offenbar ***wirklich*** geht - die (zumindest partielle) Leugnung der Corona-Pandemie. Abgesehen davon geht das Argument völlig ins Leere: Der Corman-Drosten qPCR-Test tut nicht mehr und nicht weniger als das Virus nachzuweisen, ebenso wie Varianten und Weiterentwicklungen des Tests, die seit Januar 2020 in Gebrauch sind. Maßnahmen wie Lockdown hingegen werden von der Politik entschieden, auf der Basis epidemiologischer Daten.

That’s a remarkable comment. Such aspects usually are not components of a scientific paper. As it seems, here lies the ***true*** reason for this “peer review” - the (at least partial) denegation of the ongoing Corona pandemic. Besides this, the argument completely fails: The Corman-Drosten qPCR test achieves nothing than to detect the virus - as all the derivatives of the test developed since then do. Sanctions like 'lockdown' are ordered by government on the basis of epidemiologic data.

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.

So waren schlicht die Gegebenheiten: Zu Beginn einer Pandemie ist die zur Verfügung stehende Sequenzinformation begrenzt. ***Das wird laufend nachgebessert***. Bei SARS-CoV-2 ist es so, bei HIV ist es seit Jahrzehnten nicht anders. Würden wir stets warten, bis die vollständige Sequenzinformation vorliegt (was allein wegen der sich kontinuierlich anhäufenden Mutationen gar nicht sein kann), kämen wir nie zu einem Test: Genetische Variabilität kann man erst im Laufe einer Epidemie / Pandemie kartieren. Die Relevanz etwaiger Mutationen für die Funktionalität der Primer / Sonden muss also kontinuierlich überwacht und ggf. angepasst werden, was ja auch geschieht.

Abgesehen davon sind diese Behauptungen inkorrekt:

- berücksichtigt wurden 375 Corona-Sequenzen aus der Datenbank - v.a. anderer Coronaviren, um die falschpositive Bindung der Primer an anderen Corona-Vertretern zu verhindern und dem Assay somit eine hohe Spezifität zu verleihen.
- Die Autoren geben Positiv- und Negativkontrollen an, die sie selber auf Spezifität und Sensitivität ausgetestet haben.
- Authentische RNA wurde (per in vitro Synthese) produziert und ausgetestet.
- Mit Hilfe des RdRp-Assays kann SARS-CoV-1 von SARS-CoV-2 unterschieden werden, wie die Autoren ausführlich beschreiben.

Warum wird dies von Borger & Co verschwiegen? - Das Verschweigen relevanter Information zur Bekräftigung falscher Aussagen ist ein eklatanter Verstoß gegen alle *Regeln guter wissenschaftlicher Praxis* und ebenso schlimm wie die gezielte Manipulation. Dies disqualifiziert die Autoren als Wissenschaftler.

This was just the way it was in the first months in 2020: The available sequence information was limited as is always is at the beginning of a pandemic. But developing a test system you've got no choice: ***Take what you have and improve the system as additional sequences come up***. This is how it works with SARS-CoV-2 as it has been for HIV since decades. If we decided to wait until complete sequence information is available (that will never happen due to continuously accumulating mutations) we would never get to a test: Genetic variability can be mapped only during ongoing pandemic and not beforehand. So, upcoming sequence variants have th be monitored continuously - and this is exactly what happens.

Besides this, the allegations are incorrect:

- 375 Corona sequences from public databases have been reconsidered, especially those of other corona viruses in order to avoid false positive binding of primers and probes hence achieving high specificity of the test.
- The authors give detailed information on positive and negative controls which have been thoroughly tested by themselves for specificity and sensitivity.
- authentic RNA was produced (via in vitro synthesis) and tested.
- Distinction of SARS-CoV-1 and SARS-CoV-2 is possible, as the authors show, employing the developed RdRp-assays. All details are given in the paper.

Why do Borger & Co keep quiet about this? Concealment of relevant information in order to strengthen false claims is as serious as purposeful manipulation of data. It is a severe violation of *good scientific practise*. In doing so, the authors disqualify themselves as scientists.

Das Verschweigen relevanter Information zur Bekräftigung falscher Aussagen ist ein eklatanter Verstoß gegen alle *Regeln guter wissenschaftlicher Praxis* und ebenso schlimm wie die gezielte Manipulation. Dies disqualifiziert die Autoren als Wissenschaftler.

To date no validation has been performed by the authorship based on isolated SARS-CoV-2 viruses or full length RNA thereof.

Das ist, wenn man es ***so*** formuliert, Unsinn. Der Test wird seit Monaten angewendet und allein dadurch unter härtesten (nämlich realen) Bedingungen überprüft.

In ***this*** wording, it is just nonsense. This qPCR test is in use since months and hence is tested under rigorous conditions.

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.

So ausgedrückt ist es schlicht falsch: Drosten und Koautoren beschreiben eingehend, was sie als Positivkontrollen (Plural!) verwendet haben. Darüber hinaus wäre dies alles allein schon wegen der mittlerweile erwiesenen Praxis-Tauglichkeit (inklusive Spezifität und Sensitivität) hinfällig.

In ***this*** wording, it is simply wrong. Drosten and coauthors have described some positive controls (plural!). Moreover, these arguments are obsolete because of the test's practical applicability (including the meanwhile corroborated sensitivity and specificity).

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.

Auch hier geht wieder einiges durcheinander. Dass SARS-CoV-2 (Ursache für Covid-19) ähnlich SARS-CoV-1 (aus dem Jahr 2003) ist, musste man nicht "vermuten". Das ist ***Fakt***, wie jeder, der bioinformatische Grundkenntnisse hat, selber überprüfen kann (BLAST, multiple Alignments, tree calculation). Verwendet wurde die seinerzeit verfügbare SARS-CoV-2-Sequenzinformation - was, bitte, hätte man sonst tun sollen?

Here, some aspects are mixed up again. The similarity between SARS-CoV-2 (2019/20) and SARS-CoV-1 (2003) is no "assumption" but a simple ***matter of fact***, as bioinformatic analysis clearly has told. By the way: Anybody who is somewhat familiar with bioinformatic sequence analysis can check this by BLASTing, multiple alignments and tree calculation employing freely available software. For the analysis, the Drosten group took the sequence information available at this time - what else should they have done?

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)

Das ist falsch: Die Ausgangs-Konzentration für Standard-PCR-Primer ist 1µM; bei qPCR um Faktor 2 bis 5 darunter: Geht man die Publikationen der letzten 10-15 Jahre durch und schaut sich zudem qPCR Test-Kits an, so liegt die Primer-

Konzentration mehrheitlich im Bereich 500 nM +/- 200 nM, Sonden 200 nM +/- 100 nM. Ausreißer nach oben gibt es häufiger, nach unten eher selten. Davon ausgehend ist während der Testetablierung die Konzentration nach oben und unten auszutesten. Die optimale Konzentration ist also nicht vorhersagbar, sondern muss in Form einer "Primer-Matrix" ermittelt werden. Und bei einer gut durchgetesteten und optimierten qPCR liegen die Konzentrationen der Primer und Sonden am Ende in aller Regel um Faktor 2-4 auseinander. Anders gesagt: Die Aussage "optimal = 100 - 200 nM" ist schlicht inkorrekt. Die Autoren hätten nur anhand von ***Labortests*** zeigen können, dass die Konzentration untauglich sei. Hier mit theoretischen Einwänden eine empirische Arbeit zu kritisieren, ist kein taugliches Verfahren. Im Gegenteil zeigt die mittlerweile fast einjährige Erfahrung mit dem Test, dass die Konzentrationen im Corman-Drosten-Test in Ordnung sind.

Wrong: Concentrations for PCR primers to start with in a standard PCR reaction are 1µM each and 2 to 5 times less for qPCR. Screening the literature of the last decade and comparison of commercially available kits yields mean concentratons for primers of 500 nM +/-+ 200nM and 200 nM +/- 100 nM for probes. Deviations to higher concentrations are more common than to lower ones. From this starting point usually an optimisation of concentration values - a "primer matrix experiment" - is conducted. The optima cannot be predicted / calculated, it is a game of trial and error. In a thoroughly optimised and tested PCR primer concentrations often deviate by 2x to 4x. In other words: the apodictic statement "100 - 200 nM = optimal" is incorrect, and Borger et al. only could have demonstrated by laboratory experiments that the parameters given in the Corman-Drosten paper are inappropriate. In contrary, the experience over meanwhile roughly one year shows that the concentrations given in the Corman-Drosten paper are appropriate.

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

Das ist komplizierter als hier dargestellt: Die ***Kombination*** von 2 Primern und 1 Sonde muss spezifisch sein.

It's more complicated than this - the ***combination*** of two primers and one probe has to be specific.

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

Das ist wünschenswert, aber kein "Muss" (z.B. PCR nach Bisulfit-Konversion funktioniert auch mit sehr AT-reichen Primerbindestellen).

This would be optimal but it's not a "must". (e.g. PCR with AT-rich primers after bisulfite conversion works well).

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

Falsch; es hängt von der Spezifität des qPCR-Systems ab. SYBR-green qPCR ist weniger spezifisch als eine TaqMan-PCR, bei der die im Amplikon bindende Sonde einen weiteren Spezifitätsfilter darstellt. Abgesehen davon sagt die WHO klipp und klar, dass zwei detektierte Stellen im SRAS-CoV-2 Genom ausreichen

(https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2), hier machen Borger et al. also eine Falschaussage.

Wrong - it depends on the specificity of the qPCR-systems used. SYBR-green qPCR is less specific than TaqMan qPCR, in which a hybridisation probe in the target amplicon provides additional specificity. Besides this, WHO clearly states that two detected targets in the SARS-CoV-2 genome are sufficient (https://www.who.int/publications/i/item/diagnostic-testing-for-sars-cov-2), Borger et al. make a false statement.

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℃ per primer pair).

Das ist in der Tat das Ziel, aber die Realität (die ermittelte und zu erwartende Konservierung der Zielsequenz) bestimmt das Machbare. Übrigens: Die reale Schmelztemperatur hängt von der Primerkonzentration (!), der Salzkonzentration u.a. ab; allein darum ist es schon sinnvoll, zwar mit Standard-Konzentrationen zu beginnen, dann jedoch davon ausgehend eine Optimierung der Konzentrationen vorzunehmen.

This, indeed, is the aim. However, reality (and especially sequence conservation) dictates the outcome to some extent. By the way: the real melting temperature also depends on primer concentration (!) and salt concentration - already for this reason a primer matrix optimisation experiment makes sense.

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]

Unsinn, weil gänzlich irrelevant: qPCR ist keine Endpunkt-Bestimmung, sondern es wird das Anwachsen des Amplifikats in Echtzeit (real time PCR) verfolgt. Eine mitgeführte Standard-Reihe dient als Referenz, die klar die Empfindlichkeit des Systems zeigt und auch beurteilen lässt, welchen Ergebnissen (Ct-Werten) man noch trauen kann und welchen nicht. Abgesehen davon führen falsche Probennahme oder ungünstige Probenlagerung (via RNA-Abbau) zu schwachen qPCR-Signalen (genauer: hohe und uneindeutige Ct-Werte) - es ist gut, auch solche Fälle sichtbar zu machen.

Nonsense, because completely irrelevant: qPCR is not an end-point assay but a real-time-assay. qPCR assays always are performed in comparison to a standard (usually to a dilution series) which will clearly show the sensitivity of the system. Hence it immediately can be seen which Ct-values can be trusted. Besides this, wrong

sampling or inappropriate sample storage will cause ambiguous qPCR signals (precisely spoken high and ambiguous Ct-values). It's a good thing to detect also such borderline cases.

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

Das ist vor allem notwendig bei SYBR-green qPCR-Assays: Hier kann es durch Nebenprodukte sowie Primer-Dimere zu falsch-positivem Signal kommen. Bei TaqMan PCR-Systeme ist dies durch die im Amplikon bindende Sonde faktisch ausgeschlossen. Dass in der Anfangsphase der Etablierung ***selbstverständlich*** eine gelelektrophoretische Analyse erfolgt, ist trivial und bedarf keiner Erwähnung! Auch das ist Basiswissen qPCR. Ferner unterschlagen die Autoren, dass an der Validierung mehrere Laboratorien beteiligt waren und dass die Ergebnisse in der Drosten-Publikation benannt werden.

This is necessary especially for SYBR green qPCR systems - here, false positive results may arise from insufficient primer specificity and / or primer dimer formation. For TaqMan PCR systems, however, this can in fact be excluded because of the additional stringency of the test provided by the internal TaqMan probe. ***Of course*** at the very beginning of test validation agarose gel electrophoresis is performed! This is trivial and so it needs no mention. That, too, is basic PCR knowledge. Moreover, the authors keep quiet about the fact that some laboratories (and not only the Drosten lab) participated in the validation process and that the results are outlined in the paper.

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

Klassische ***Negativkontrollen*** müssen nicht spezifiziert werden, sie enthalten schlichtweg kein Template. Das ist Basiswissen PCR. Daneben listen die Autoren einige Templates auf, die als ***Spezifitätskontrollen*** genutzt werden können, und als ***Positivkontrolle*** kann SARS-CoV strain Frankfurt-1 dienen. Und um die Kreuzreaktivität mit anderen Viren auszuschließen, testeten die Autoren 297 klinische Proben, die mit insgesamt gut 20 verschiedenen respiratorischen Erregern faktisch das gesamte Spektrum abdecken. Auch dies verschweigen die Autoren.

Classical ***negative controls*** need not to be specified - they just contain no template. This is basic PCR knowledge. Moreover, the authors list a couple of templates that can be used for ***specificity control***. As ***positive control***, SARS-CoV strain Frankfurt-1 virus is suitable. Cross reactivity with other respiratory viruses was tested with 297 clinical samples containing over 20 different respiratory pathogens and hence covering the complete spectrum of pathogens to be expected in this context.

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.

Das ist inkorrekt. SOPs gelten in ***jeweils einem konkreten*** Labor, weil sie den dortigen Bedingungen (Apparaten, Räumlichkeiten etc.) angepasst werden, ergo werden sie in jeder Arbeitsgruppe entwickelt oder zumindest angepasst. Das

vorgelegte Corman-Drosten-Protokoll ist vielmehr eine Richtlinie, damit Anwender ihre eigene SOP etablieren können.

This is incorrect. SOPs are specific ***for a given lab / working group*** because they are adapted to the given conditions (devices, rooms / ambience etc.). The Corman-Drosten protocol in fact is a guideline so that users can define their own SOPs.

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.

Stimmt, das ist ein Tippfehler. Das sollte nicht sein, kann aber vorkommen. Allerdings steht in der Legende explizit, dass die Konzentrationen in nM angegeben sind.

Yep, it’s a typo. Shit happens. However, the legend clearly states that concentrations are given in nM.

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.

Jeder, der Erfahrung mit PCR-Systemen hat, weiß: "R" und "Y" sind Standard-IUPAC-Bezeichnungen für die beiden Purin- bzw. Pyrimidin-Basen. Und ist ***vollkommen üblich***, in einem Alignment die Reverse-Primer auch in passender (also komplementärer) Orientierung anzugeben.

Everyone who is familiar with PCRF systems knows: "R" and "Y" are standard IUPAC code letters for purines and pyrimidines, respectively. And it is ***standard procedure*** to note reverse primers in an alignment in adapted - that is, reverse complement - orientation.

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).

Tm-Werte hängen vom Puffer (insbesondere der Mg^{2+}-Konzentration) und der Primerkonzentration ab. Man kann sie mit im Internet frei verfügbaren Tools berechnen - den GC-Gehalt ebenfalls (oder man zählt ihn einfach in WORD aus...). Kurz: Wer etwas von PCR versteht, kann die Werte selber und passend für das eigene System berechnen. Darum werden sie in einer Publikation auch nur selten angegeben.

Tm-values depend on buffer composition (especially Mg^{++} concentration). They can easily be calculated employing tools freely available on the internet - GC content, too (or you simply count it out by yourself using a WORD processor). In other words: Everyone who is familiar with PCR can calculate these values by himself for the own PCR-system. This is why GC content rarely are mentioned in publications.

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?

Angesichts solchen Unsinns stockt der Atem, denn erstens gibt es infektiologische Parameter, anhand derer man abschätzen kann, wie gefährlich ein Erreger sein mag. Und zweitens ist es angesichts von knapp 30.000 Todesopfern in Deutschland und etwa 1,7 Millionen weltweit (Ende 2020) mittlerweile gegenstandslos, diese Frage überhaupt zu erörtern.

This is just breath-taking nonsense. First, there are infectiological parameters which are suitable to assess the future risk of a given pathogen. Second, in view of meanwhile 1.7 million victims worldwide (by the end of 2020) it is absolutely meaningless to discuss this question.

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.**

Hier zeigt sich am deutlichsten, dass die Autoren offenbar keinerlei eigene Erfahrung mit qPCR und darüber hinaus auch keine theoretische Sachkenntnis haben: Primer-Konzentrationen kann man nicht vorherberechnen, man muss sie für jedes PCR-System erneut empirisch ermitteln. Dabei ist auch Sensitivität und Spezifität zu testen, was Drosten und Mitautoren umfassend getan haben.

This point clearly shows that the authors obviously are unfamiliar with qPCR and moreover do not possess technical knowledge: Primer concentrations cannot be calculated beforehand, they have to be determined empirically. Concomitantly, specificity and sensitivity have to be tested, and this has been done thoroughly by Drosten and co-authors.

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!

Die Buchstaben W, S, M, K, R, Y sind Standard-Bezeichnungen aus dem IUPAC-Code für Nukleinbasen - in diesem Fall für jeweils zwei der vier möglichen. Das ist ***Basiswissen*** für einen jeden PCR-Experten. Daher ist es Unsinn zu behaupten, dies wäre verwirrend für die Anwender. Weiterhin ist es unsinnig, definierte Sequenzen für Primer und Sonden zu fordern: Die reale Konservierung der Sequenzen (also die Ähnlichkeit der zirkulierenden [Sub-]Stämme) diktiert die Sequenzen der Primer und Sonden und entscheidet darüber, ob sie eindeutig oder degeneriert sind. Im Übrigen verstehen die Autoren die Logik degenerierter Primer und Sonden ganz offensichtlich nicht, denn hier gibt es mitnichten 64 Kombinationen: Degenerierte Primer und Sonden werden nicht als verschiedene Chargen, sondern direkt als Gemisch synthetisiert und eingesetzt. Auch das ist Basiswissen.

The letters W, S, M, K, R, and Y are standard IUPAC symbols for nucleobases, these symbols represent two distinct bases out of four. This is ***basic knowledge*** for every PCR expert. Hence it is sheer nonsense to criticise this as "confusing for applicants". It is the conservation of the sequences (i.e., sequence similarity of circulation [sub]-

strains) that decide if distinct primer and probe sequences can be designed unambiguously or if they have to be degenerate. Moreover, the authors obviously do not understand the logic of degenerate primers and probes, for there are no "64 possible combinations of primers and probes". The different versions are not, as the authors obviously believe, synthesised separately. They are synthesised from the start as a mixture and used as such. This is basic knowledge, too.

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

Die Spezifität eines Nachweises hängt von mehreren Faktoren ab. Zwei unabhängige PCR-Systeme - hier zuerst das E-Gen, danach das RdRp-Gen - stellen bereits einen recht guten Nachweis dar. Noch besser ist allerdings die Tatsache, dass es sich um TaqMan-Systeme handelt, bei denen - wie erwähnt - jeweils drei Oligonukleotide unabhängig voneinander spezifisch binden müssen, damit der Nachweis positiv ist. Ergo sind hier zwei Targets hinreichend. Dabei ist es unerheblich, wo im Virus-Genom diese Targets sitzen: Hauptsache, sie sind unabhängig voneinander.

The specificity of an assay depends on several aspects. Two distinct and independent PCR systems already are not bad. With two qPCR systems, specificity is even further improved by the fact that a TaqMan qPCR system is used: Here, for each PCR assay three oligonucleotides (rather than two) have to bind specifically and independent from each other to yield a positive result. In this context it does not matter at all where amplification targets are located in the viral genome - given, they are independent from each other.

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.

Hier geht einiges durcheinander, nämliche Diagnose der Erkrankung und Nachweis des Erregers. Die Erkrankung wird über Anamnese diagnostiziert, der Erreger mikro-biologisch / virologisch (Anzucht) oder molekular. Eine Anzucht dauert lang und war in den ersten Monaten der Pandemie als Nachweismethode praktisch nicht möglich; Antigentests gab es ebenfalls nicht. Und Goldstandard bei Virusnachweisen ist heut-zutage die qPCR (z.B. für HIV ist Nachweis und Quantifizierung über ELISA oder qPCR aus Blutserum der aktuelle Standard!). Vorteil des qPCR-Assays ist erstens seine Sensitivität und zweitens die Tatsache, dass die Viruslast damit quantifizierbar ist. As diesen Gründen geht das nachstehende Zitat ebenfalls komplett am Thema vorbei.

Some aspects are mixed up here, namely diagnosis of the disease and detection of the pathogen. Diseases are diagnosed via anamnesis. Pathogens are identified by cultivation or by molecular biology methods. Cultivation takes too long and moreover was in fact not possible in the first months of SARS-CoV-2 pandemia - at least not as a detection tool. Antigen tests were not available. Moreover, contemporary gold standard is qPCR (e.g. for HIV detection in patients' blood by ELISA or qPCR is state of the art since decades). Advantage of the Drosten qPCR system lies (i) in its sensitivity (based on the TaqMan principle) and (ii) in the opportunity to quantify the virus load. By the way: Detection and quantification of HIV in blood (serum) by qPCR is state of the art... And for these reasons, the following citation is also off-topic.

“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).

Nochmals: Zwei TaqMan-qPCR-Systeme sind (mindestens!) so gut wie drei Standard-PCR-Systeme.

Once again: Two TaqMan qPCR systems are (at least!) as good as three standard PCR systems.

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.

Es ist irrelevant, dass die Amplikons "nur ein halbes Virusgenom auseinander" liegen. Hauptsache, unterschiedliche Gene werden nachgewiesen.

It is absolutely irrelevant that the two qPCR amplicons are separated by "only half a viral genome" - given that distinct genes are targeted.

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.

Auch hier zeigt sich die Unkenntnis der Autoren.
1.: Oftmals ist es gar nicht möglich, Primer an die Genom-Enden zu setzen, denn die Konservierung der Sequenzen diktiert die Möglichkeiten des Primer-Designs.
2.: Die Unterscheidung von kompletten und fragmentierten Genomen ist unnötig: Ein fragmentiertes Virus-Genom entstammt einer Zelle, die mit einem intakten Virus infiziert war, und daher zeigt ein defektes Virus-Genom ebenso eine Infektion an wie ein intaktes.

This again shows the ignorance of the authors.
1.: It is often impossible to put primers to the ends of the target viral genome - it is the sequence conservation that dictates possible primer target sites.
2.: The distinction of complete and fragmented viral genomes is unnecessary: A fragmented viral genome, too, originated from a cell infected by an intact virus. This it also indicates an infection.

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].

Auch hier liegt seitens der Autoren ein grobes Missverständnis vor:

- Welche Teile des Genoms wie stark exprimiert werden, ist für den Nachweis weniger relevant, denn nachgewiesen werden vor allem Viruspartikel, in denen das Virusgenom vorliegt - bei den meisten Viren genau eine Kopie (bei manchen genau zweie). Unterschiedliche Expressionsraten wirken sich also weniger aus als hier offenbar vorausgesetzt.
- Es ist sowohl sinnlos als auch unfair, mit Kim et al. ein Paper zu zitieren, das erst Monate ***nach*** dem Dosten-Paper veröffentlicht wurde.

This, again, is a misunderstanding:

- Viral expression profiles and differences are less relevant for virus detection, because mainly virus particles (containing the viral genome) are detected. And a virus particle contains one genome (some viruses two) with exactly one copy of each gene. Hence, different expression rates do not have too much effect.
- It is both meaningless as well as unfair to cite the paper of Kim which was published some months ***after*** the Drosten paper.

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.

Hier liegt ein schwerer Fehler vor (siehe unten).

This is a severe mistake (see below).

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).

Das ist völlig irrelevant: Für eine erfolgreiche PCR müssen beide Primer passen, für eine erfolgreiche TaqMan-qPCR alle drei Oligonukleotide. Das ist Basiswissen PCR.

That's completely irrelevant. For successful PCR amplification, both primers have to bind stably, for a successful TaqMan qPCR even all three oligonucleotides. This is basic PCR knowledge.

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];

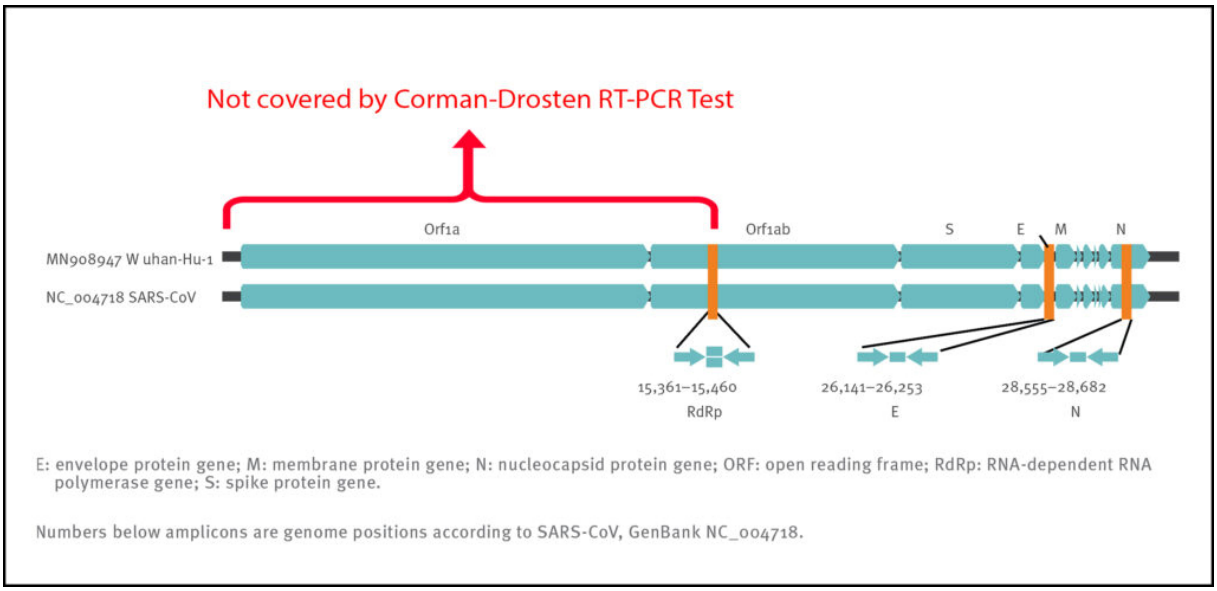
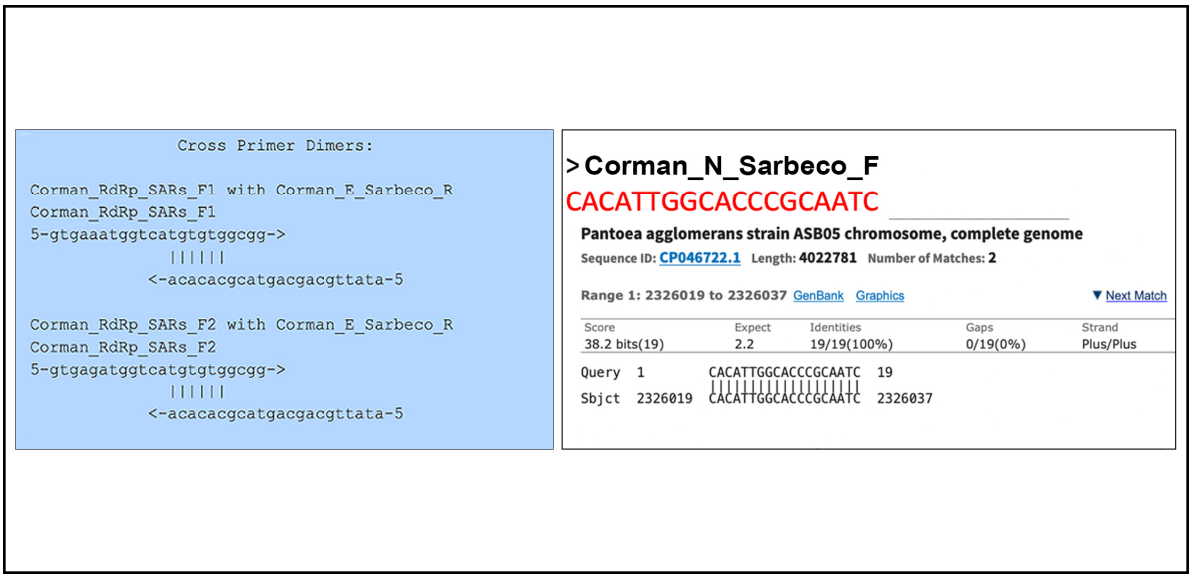


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (Pantoea) found in immuno-compromised patients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



Hier ist den Autoren ein schwerer Fehler unterlaufen:
E-Gen Primer und RdRp-Primer werden nicht kombiniert, sondern nacheinander in voneinander unabhängigen Test eingesetzt, kommen also nie zusammen - das steht klar im Paper, man muss es nur lesen...

A severe mistake:
The Corman-Drosten paper clearly states that the two qPCR systems - E gene and

RdRp - have to be used independently from each other - the two respective primers never are used together. One should simply read the paper before uttering criticism...

2. Reaction temperatures

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.

1.: Diese angebliche Unspezifität ist eine bloße Behauptung der Autoren ohne jede Untermauerung, noch nicht einmal durch BLASTen der Sequenzen. Im Gegensatz dazu haben Drosten und Koautoren ausführliche Tests betreffend Sensitivität und Spezifität durchgeführt, die sie im Paper auch darlegen. Warum verschweigen dies die Autoren?
2.: Wenn ein Primer-Schmelzpunkt niedrig ist und die Annealing-Temperatur mit 58°C relativ hoch, so ***erhöht*** das sie Spezifität. Auch das ist PCR-Grundwissen.
1.: Unspecific binding is just a claim of the authors without any corroboration, not even by BLASTing of the sequences. On the other hand, Drosten and colleagues have tested sensitivity and specificity of their PCR system thoroughly. Why keep the authors quiet on this?
2.: If the melting temperature is quite low but a rather high annealing temperature is use, this will ***increase*** specificity. This again is basic PCR knowledge.

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.**

Richtig ist, dass die (berechneten!) Schmelzpunkte der Primer weiter auseinander liegen als üblich. Allerdings verschweigen die Autoren, dass die Annealing-Temperatur mit 58°C recht hoch und somit stringent ist.

Indeed, difference between the (calculated!) melting temperatures are greater than usual. However, the authors do not mention that annealing temperature is 58°C and hence quite stringent.

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.

Noch ein grober Anfänger-Fehler: 0,4 µM ist dNTP-Konzentration im Mastermix, der das halbe Volumen des Ansatzes ausmacht, so dass die finale Konzentration genau den üblichen 0,2 µM entspricht. Das Berechnen von Ansätzen mit Mastermixen lernen Studierende bereits in den ersten zwei Semestern.

This is one more beginner's mistake. 0.4 µM is the dNTP concentration in the PCR master mix. This mix accounts for half of the final volume of the PCR sample so that the final dNTP concentration equals exactly the recommended 0.2 µM. Our students learn in their first semesters how to calculate samples employing a master mix!

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.

Erstaunlich, dass dies nach mehr als einem Dreivierteljahr Anwendung weltweit noch niemandem aufgefallen ist, dass jedoch Borger et al. dies erkennen können, ohne auch nur einen einzigen Versuch im Labor durchgeführt zu haben.

Surprising, that no one has realised this so far - after more than half a year application worldwide. And even more surprising, that Borger et al. could identify all those "flaws" without running a single laboratory experiment.

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.

Das Drosten-Paper benennt mehrere Kontrollen.

In the Drosten-Paper, quite a few controls are defined.

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.**

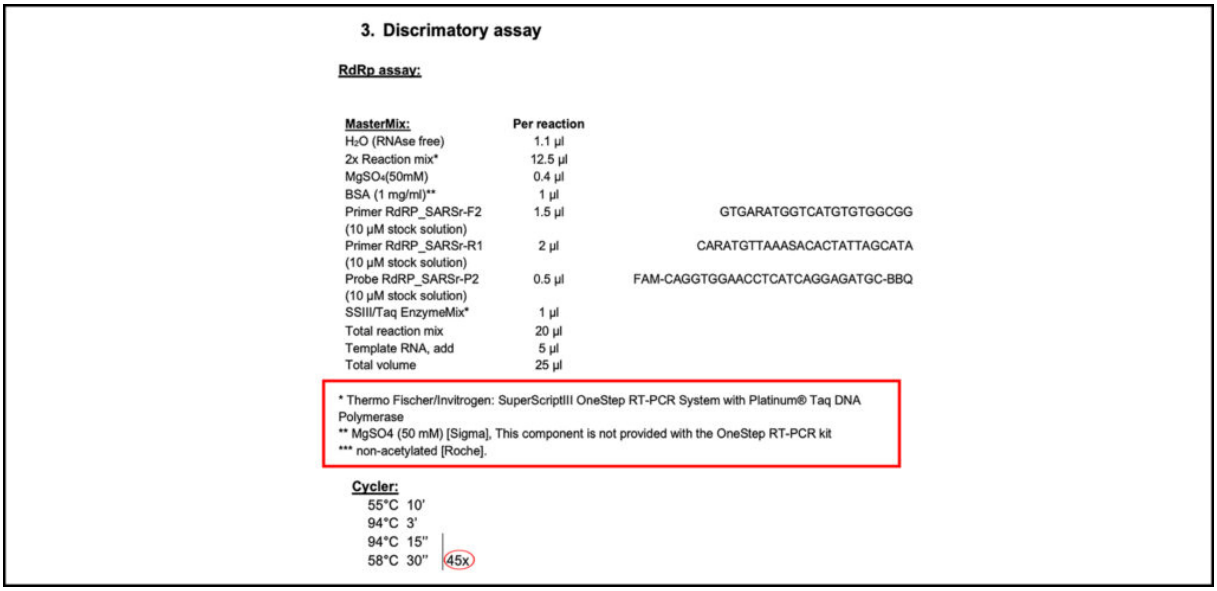
Further, scientific studies show that only non-infectious (dead) viruses are detected with Ct values of 35 [22].

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.

Jeder, der jemals qPCR selbst durchgeführt hat, weiß: In einer qPCR werden weit mehr Zyklen gefahren als nötig (so bekommt man übrigens einen guten Eindruck über die Spezifität des Systems). Parallel zu den zu testenden Proben läuft eine Kalibrierreihe mit. Letztlich definiert man in jedem neuen PCR-Lauf eine Schwelle ("threshold"), die vom Signal überschritten werden muss. Dies erlaubt dann einen Vergleich zwischen der Kalibrierreihe und den zu analysierenden Proben. Diese Schwelle hängt u.a. auch vom qPCR-Apparat ab, so dass es unsinnig ist, diese Schwelle im Paper zu definieren, wie es die Autoren fordern. Und wieder: qPCR-Grundwissen.

Everyone who has ever conducted qPCR by himself knows: qPCR is performed in many more cycles than needed (which, by the way, permits assessment of specificity). Concomitantly to the samples to be analysed, a series of standards is processed. After the PCR procedure, a threshold is defined (for each and every run) which allows comparison of the standards to the samples. This threshold depends - amongst others - on the qPCR device used. Hence it does not make any sense to define it in a protocol or a paper. And again: basic knowledge qPCR.

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.

Nebenbei: Ethidiumbromid (EtBr) wird wegen seiner potenziellen Kanzerogenität praktisch nicht mehr verwendet, seit über 10 Jahren gibt es mindergiftige Alternativen. Jeder, der noch mit praktischer Laborarbeit zu tun hat, weiß das... Was die Amplifikate anbelangt, so ist ***selbstverständlich*** eine gelelektrophoretische Kontrolle erfolgt! Deren Dokumentation ist jedoch angesichts der sehr hohen Spezifität einer TaqMan-qPCR verzichtbar.

By the way: Ethidium bromide (EtBr) hardly is used any more due to its (potentially) carcinogenic properties. Alternatives of reduced toxicity exist since more than one decade. Everyone who is still in touch with laboratory work knows this. Concerning the amplificates - Drosten and Coworkers ***of course*** have performed gelelectrophoretic analysis! But due to the very specificity of TaqMan-qPCR it is unnecessary to document the results.

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.

Das ergibt keinen Sinn.

This does not make any sense.

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.

Aus den benannten Gründen (siehe oben) ist dies falsch.

For the reasons discussed above this is wrong.

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.

So ausgedrückt ist das falsch. Bekanntermaßen zirkulieren in der Menschheit bereits mehrere Corona-Stämme, die grippale Infekte verursachen. Von den SARS-like beta-Coronaviren zirkuliert nur SARS-CoV-2, für alle anderen inklusive SARS-1 gibt es derzeit keine Hinweise auf Infektionen beim Menschen. Andere Beta-CoV (HKU & OC43) zirkulieren natürlich und wurden getestet. Die Behauptung, es gäbe "unconfirmed assumption", ist also falsch. Seit 2003 werden die SARS-like beta-Coronaviren global überwacht und es werden regelmäßig neue Stämme (in Fledermäusen und anderen Säugetieren) entdeckt - die sind aber bis Ende 2019 nicht wieder im Menschen aufgetaucht. SARS-CoV-2 ist also das einzige Virus, das für die derzeitige Pandemie verantwortlich ist. Im Corman-Drosten-Paper ist ausführlich beschrieben, gegen welche respiratorischen Viren das qPCR-System umfassend getestet wurde, darunter auch vier Corona-Stämme.

In this wording it is wrong. It is well known that several coronavirus strains circulate in mankind cause common cold. However, of SARS-like ß Corona viruses currently only SARS-CoV-2 is circulation in mankind. Other circulating ß corona viruses (HKU, OC43) have been tested. Since 2003 SARS-like ß corona viruses are monitored worldwide and quite often novel strains are detected in bats and other mammals. None of them have appeared in humans so far. Hence "unconfirmed assumption" simply is wrong. The Corman-Drosten paper describes in detail the testing of the developed qPCR system against other respiratory viruses, among these four corona strains.

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].

Erstens gab es zu dieser Zeit keine andere Möglichkeit. Wollte man warten, bis Sequenzinformationen aller möglichen Stämme verfügbar sind (was alleine schon wegen der sich anhäufenden Mutationen unmöglich ist), käme man nie zu einem Test. Zweitens wird man diese Tests ständig nachbessern also neu verfügbaren Sequenzinformationen anpassen - so wie bei HIV-Tests seit Jahrzehnten. Drittens

hat sich das Corman-Drosten-pPCR-System bewährt, was Borger et al. eigentlich bekannt sein dürfte.

First, there was no alternative in January 2020 - you have to take what you can get. It makes no sense to wait until sequence information of all possible strains is available (which cannot happen anyway due to accumulating mutations). Second, it is routine to continuously improve existing tests and adapt it to novel sequence information. Third, given the success if the Drosten qPCR system (Borger et al. should know!), it is futile to argue against it.

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.**

Hier liegt ein schweres Missverständnis vor: Virales Erbmaterial in einem Patienten-Abstrich - im Falle von Corona-Viren also virale RNA - wird nicht "einfach so" vom Körper gebildet, sondern entsteht ausschließlich im Zuge einer Infektion. Es ist also völlig egal, wie viele der nachgewiesenen Kopien intakt sind und wie viele defekt: Sie wurden allesamt von Virus-infizierten Zellen des Probanden erzeugt und zeigen somit eine aktive Infektion an (ob die betreffende Person nun erkrankt oder nicht) - im Falle des Drosten qPCR-Systems also eine SARS-CoV-2 Infektion. Wie sollte denn sonst virale RNA in einen Abstrich kommen? Allein der Übertrag (defekter) Virus-partikel durch Aerosol liegt unter der Nachweisschwelle, wird also nicht detektiert. Ein deutlich positiver Test bedeutet somit automatisch eine aktive Infektion - mit oder ohne dadurch ausgelöste Erkrankung. Das ist virologisches Grundwissen, dieser grobe Fehler hätte zumindest Prof. Ohashi - dem einzigen Virologen aus dem Autoren-team (!) - auffallen müssen.

This is a server misunderstanding. Viral nucleic acids in a swab cannot have been produced "just so" by the patient. It will always be produced in the course of an active infection. Hence it does not matter how many of the detected copies are intact or defective. They all have been produced by virus infected cells of the patient and hence they indicate an active infection (no matter if the respective person develops the disease or not) - in the case of the Drosten qPCR system by SARS-CoV-2. How else should copies of viral genetic material have come into a swab? Inhalation of aerosol with only defective virus particles will not yield a titer high enough for detection. Hence, a clearly positive test confirms infection - with or without triggered disease. This is basic knowledge in virology and at least Prof. Ohashi - the only (!) virologist among the authors - should have recognised this.

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.

Das ist schlicht falsch - Positivkontrollen sind in der Corman-Drosten Publikation benannt - insbesondere SARS-CoV-1 ist perfekt geeignet.

This is simply wrong. The Corman-Drosten paper notes positive controls - especially SARS-CoV-1 is very well suited.

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.

Hier liegt ein schwerwiegender Irrtum von Borger et al. vor - das E-Gen kommt bei allen Corona-Viren vor! Es gibt kein einziges Gen, das für SARS-CoV-2 spezifisch wäre. Es geht also um Sequenzanteile des Genoms, die für SARS-CoV-2 spezifisch und gleichzeitig konserviert sind. Es geht also darum, Sequenzvarianten zu detektieren, die sich bei SARS-CoV-2 (und eng verwandtet Viren) finden lassen, nicht aber bei allen anderen Coronaviren, die z.Zt. in der Menschheit zirkulieren. Und genau das leistet der Test.

This is a severe misunderstanding by Borger et al. The E gene exists in all corona virus strains. There is not a single gene which is specific for SARS-CoV-2. The task is to find sequence parts in the genome of a virus which are both specific for the viral taxon of interest as well as sufficiently conserved. For this reason the task is to detect sequence variants present in SARS-CoV-2 (and close relatives) but absent in all other viruses circulating in mankind to date. And this is exactly that the test achieves.

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

Richtig. Aber außer SARS-CoV-2 keinen einzigen weiteren Stamm, der z.Zt. in der Menschheit zirkuliert.

Yes. But except for SARS-CoV-2 not a single other one circulating in mankind to date.

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].**

Jeder, der einmal einen PCR- oder qPCR-Test designt hat, weiß: Man designt und probiert verschiedene Primer-Kombinationen und verwendet am Ende diejenigen, die am besten "laufen". Da die Design-Möglichkeiten schier unerschöpflich sind, werden am Ende keine zwei Arbeitsgruppen exakt dieselben PCR-Systeme konstruiert haben. Daher ist es müßig zu räsonieren, welche anderen Primer-Systeme hätten verwendet werden können. Alles, was zählt, ist die Tatsache, dass der Test spezifisch ist, was sich mittlerweile auch weltweit in seiner Praxistauglichkeit zeigt.

Everyone who has ever designed PCR- oder qPCR-tests knows: You design and test quite a few primer combinations and in the end you will choose the ones which perform best. Since possible combinations of primers and probes are inexhaustible two working groups in the end never will come out with identical PCR systems. For this it is futile to grumble why amplicon X was chosen instead of amplicon Y. All that counts is specificity of the Corman-Drosten system which meanwhile has been corroborated worldwide.

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.**

Das ist schlicht unwahr: Die Corman-Drosten-Publikation benennt ausdrücklich Positiv- und Negativkontrollen.

This is simply untrue. The Corman-Drosten paper explicitly specifies positive and negative controls.

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.**

Das ist inkorrekt. Die relevanten Parameter - wie die Konzentrationen, Zyklusbedingungen der PCR - werden in der Corman-Drosten-Publikation benannt. SOPs hingegen gelten in ***jeweils einem konkreten*** Labor, weil sie den dortigen Bedingungen (Apparaten, Räumlichkeiten etc.) angepasst werden, ergo werden sie in jeder Arbeitsgruppe entwickelt oder zumindest angepasst.

This is incorrect. All relevant parameters - like concentrations and cycling conditions - are given in the Corman-Drosten paper. SOPs, however, are ***specific for a given lab / working group*** because they are adapted to the given conditions (devices, rooms / ambience etc.).

Further, the Ct value to indicate when a sample should be considered positive or negative is not specified.

Jeder, der jemals qPCR selbst durchgeführt hat, weiß, dass Nachweis bzw. Quantifizierung nach einer anderen Logik abläuft: Es ist gar nicht ***möglich***, vorab einen Ct-Wert zu spezifizieren. Tatsächlich geht man anders vor: Es wird eine Verdünnungsreihe einer definierten Positiv-Probe als Kalibrierreihe verwendet. Ein Threshold (= Schwelle) wird ***nach*** erfolgter qPCR (z. B. abhängig vom Hintergrundsignal) definiert und die Ergebnisse der Kalibrierreihe erlauben erst eine Beurteilung z. B. der unteren Detektionsgrenze. Auch dies ist Grundlagenwissen qPCR.

Everyone who ever has run qPCR knows that this is not the way the process works. It even is ***impossible*** to specify a defined Ct-value beforehand. Instead, a defined positive sample in a dilution series serves as series of standards. A threshold only can be defined ***after*** the end of the qPCR run. Now, the series of standards serves as a "ruler" which allows both quantification of samples and determination of the detection limit. This, too, is basic qPCR knowledge.

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.

Wieder ein grundlegender Irrtum. Erstens muss der Test in einem jeden Labor erneut etabliert werden. Die Ermittlung der Sensitivität erfolgt dann im Vergleich zu einer mitlaufenden Kalibrierreihe (s.o.). Zweitens ist kein einziger Test in der Lage, zwischen fragmentierten und kompletten Genomen zu unterscheiden, denn beliebige Teile eines Genoms können verloren gehen (was auch immer mal wieder passiert). Drittens entstammt auch ein fragmentiertes Virusgenom einer Zelle, die mit einem intakten Virus infiziert ist. Daher zeigt ein positiver Test in jedem Fall eine SARS-CoV-2-Infektion an.

Again a severe misunderstanding. First, this test is to be established in each and every laboratory again. Sensitivity can then be judged by comparison to the calibration series (see above). Second, there is not a single test that can tell between fragmented and complete genomes, since every part of a genome eventually can be lost (and indeed, this happens from time to time). Third, even a fragmented genome originates from a host cell infected with an intact virus. So, a positive test indicates a SARS-CoV-2 infection anyway.

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?

Alle relevanten Informationen stehen in der Corman-Drosten-Publikation. Es hätte gereicht, das einfach mal nachzulesen.

All relevant information is given in the Corman-Drosten paper. Simply read it!

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.

Das ist schlichtweg unwahr (siehe oben).

That is simply untrue - for the reasons see above.

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.

Korrekt ist im Gegenteil, dass ist der Borger-Text eine große Anzahl von Fehlern - dabei auch Anfänger-Fehler - enthält (siehe oben).

The opposite is true: The text of Borger et al. contains a lot of mistakes and errors, among these even some beginner's mistakes - see above.

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.

Angesichts der weltweiten, praktischen Erfahrung mit diesem Test über ein Dreivierteljahr ist es anmaßend und unverschämt, dessen Unzuverlässigkeit zu behaupten, ohne damit auch nur einen einzigen Test im Labor unternommen zu haben.

Given nine months of practical experience with this test worldwide is really insolent to claim unreliability of the Corman-Drosten qPCR without having performed even a single experiment with this system.

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.

Interessant, was hier zitiert wird - aber noch interessanter, was ***nicht*** zitiert wird: "In total, this testing yielded no false positive outcomes" unmittelbar vor dem zitierten Absatz. Zur Klarstellung: "weak initial reactivity" bezeichnet den Hintergrund, das Grundsignal. Das sind keine falsch-positiven Ergebnisse, wie der Borger-Text fälschlich behauptet. Abgesehen davon gibt es ja genau für den Ausschluss von eventuellen Falschpositiven den Bestätigungstest über RpRd.

The citation is interesting, but much more interesting what has ***not*** been cited by Borger et al: "In total, this testing yielded no false positive outcomes." - the sentence just before the cited passage. To clarify - "weak initial reactivity" denotes noise, background signal, but not false positive results as Borger et al. erroneously claim. By the way - the RdRp assay was designed exactly for exclusion of (eventually occurring) false positives.

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.

Das ist wieder eine bloße Behauptung ohne jede Untermauerung mit eigenen Daten. Die Autoren haben noch nicht einmal die Daten von Drosten und Mitautoren eingesehen. Im Übrigen ermöglichte gerade die frühe Publikation des Corman-Drosten-Tests den Anwendern, in Ruhe und ohne unmittelbaren Zeitdruck ihre eigene Etablierung durchzuführen und ihre eigene SOP zu definieren. Dennoch den betreffenden Abschnitt als "telltale observation" abzuqualifizieren ist schlicht unverfroren.

This again is another mere allegation without any support by own data. Borger et al. even have not seen - much less analysed - the data of Drosten and coauthors. By the way, the early publication of the Corman-Drosten test enabled users to establish the detection qPCR in their labs and to define their own SOPs without immediate pressure of time. Nevertheless disqualifying the respective part of the Corman-Drosten paper as "telltale observation" simply is impertinent and barefaced.

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].

Selbstverständlich gab es ein peer-review! Und gerade Eurosurveillance ist für sehr schnelle Begutachtung bekannt, wenn Publikationen anstehen, die als dringend betrachtet werden. Es gibt mehrere Möglichkeiten, den Begutachtungsprozess zu beschleunigen, z.B. dadurch dass die verpflichteten Gutachter an Tag und Stunde der Einreichung bereit stehen. Manchmal wird allerdings auch als Datum der Einreichung derjenige Tag angegeben, an dem die letzten Dokumente - z.B. Material, das die Gutachter nachfordern - eingereicht wurde, und das kann dann selbst verständlich der Tag sein, an dem das Manuskript eingereicht wurde. In diesem Falle stand das Manuskript der Gutachtern eine Woche vor offiziellem Einreichungstermin auf einem Preprint-Server zur Verfügung.

Of course there was a peer-review process! And especially Eurosurveillance is known for extremely fast review processes, especially when the respective publication is regarded as being urgent. There are some possibilities for acceleration of a peer review process, e.g. reviewers standing by the very day and hour when the manuscript is filed. And sometimes, day of submission is defined as the day, when the last documents were filed, e.g. information demanded in addition by the reviewers. In the case of the Corman-Drosten paper, the manuscript had been available to the reviewers on a preprint server one week before the official date of submission.

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.

Dass Editoren in ihren eigenen Journalen publizieren ist entgegen der Behauptungen nicht unüblich. Die Journale haben für solche Fälle definierte Vorgehensweisen, u.a. dass die Betreffenden aus dem Editoring- und Review-Prozess ausgeschlossen werden.

Contrary to the claims of Borger et al. it is not uncommon that editors publish in their own journals. For such cases, journals have defined procedures - namely to exclude the respective persons from editoring and peer review process.

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.

Jeder, der jemals einen PCR-Test - insbesondere solche einen qPCR-Test wie im Corman-Drosten-Paper - entworfen hat, weiß: Die Standard-Konzentrationen sind Richtwerte, mit denen man die Etablierung des Tests beginnt. Eine Voraus-berechnung der optimalen Konzentrationen ist nicht möglich: Im Rahmen der PCR-Optimierung muss man verschiedene Konzentrationen austesten. Daher liegen die finalen Konzentrationen nur selten beim Standard-Wert. Mit anderen Worten: Es ist also schon im Ansatz verquer, einen Wert, der empirisch bestimmt werden muss, theoretisch (d. h. ohne Gegentests) zu kritisieren.
Everyone who is experienced in the design of such qPCR systems knows: The so-called standard concentrations only are guide values to start with. Appropriate concentrations have to be determined empirically. Hence it is failed to criticise a qPCR only on "theoretical" grounds without any laboratory test.

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.

Hier liegen vier schwere Irrtümer von Borger und Koautoren vor:
I: Virale Genome mutieren nun einmal, und die sich ansammelnden Mutationen sind ungleich über das Virus-Genom verteilt: Es gibt Bereiche, die funktional besonders wichtig sind, und daher werden von der Selektion hier nur wenige Mutationen geduldet (diese Bereiche sind "evolutionär konserviert"). Als Zielposition für die Bindung der PCR-Primer (vgl. Punkt 1) sucht man sich diejenigen Bereiche aus, die einerseits typisch für die nachzuweisende Virengruppe sind, andererseits in eben jener Gruppe aber konserviert. Diesen Wunsch erfüllt einem die Natur aber leider äußerst selten, wie jeder weiß, der Erfahrung mit Virus-Diagnostik über PCR-Tests hat. Ergo muss man nehmen, was die Natur einem bietet, und das sind in aller Regel Sequenzabschnitte mit solchen "Wobbel-Positionen". Das ist also kein Design-Fehler, sondern eine schlichte Anpassung an die Gegebenheiten.
II: Durch diese "Wobbel-Positionen" kommen mitnichten "enorme Variabilitäten im Test" zustande. Borger und Koautoren wissen offenbar nicht, dass die betreffenden Oligonukleotide bereits als Gemisch synthetisiert, geliefert und im Test eingesetzt werden.
III: Auch die Behauptung, solche "Wobbel-Positionen" wären verwirrend für den Anwender, zeigt, dass Borger und Koautoren keinerlei Erfahrung mit qPCR-Systemen haben: So, wie jeder Kfz-Mechaniker weiß, was ein gekröpfter Ringschlüssel ist, kann jeder halbwegs routinierte PCR-Anwender "Wobbel-Positionen" lesen und verstehen.
IV: Es ist inkorrekt, dass eine PCR mit "Wobbel-Positionen" automatisch unspezifisch wird. Diese nicht spezifizierten "Wobbel-Positionen" müssen bei der Etablierung und Validierung des Tests berücksichtigt werden. Für HIV-Tests ist dieses Vorgehen seit Jahrzehnten Standard.
Aus diesen Gründen gehören Oligonukleotide mit "Wobbel-Positionen" zu Standard-Repertoire der PCR-Diagnostik auf Viren, was Borger und Koautoren offenbar nicht wissen.
Here four severe errors of Borger et al have to be stated.
I: Viral genomes accumulate mutations, not equally distributed over the genome. For primers target positions have to be chosen that are as well conserved as possible, at the same time different to other groups. However, perfect targets in fact do not exist. Hence, positions with ambiguities, resulting in wobble-positions, have to be accepted. This is proper design rather than a design error.
II: Such wobble positions do surely not create "enormous variability". Primers with wobble positions from the start are synthesised and later used as defined mixtures.
III: For users, wobble positions do not cause confusion. Every professional PCR operator knows the respective code and knows how to handle it.
IV: PCR with wobble-primers will not automatically lose specificity. Specificity is determined during establishment and validation of the test. By the way, wobble-primers are standard (not only) for HIV-diagnostics since decades.

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.

Dieses Argument ist verfehlt. Zum einen kann kein einziger Test komplette von fragmentierten Virusgenomen unterscheiden. Andererseits ist dies auch nicht nötig: Jedes virale Genom - ob komplett oder fragmentiert, ob intakt oder defekt, ob ursprüngliche Version oder mutiert - entstammt einer infizierten Zelle. Wie jeder

Infektiologe und Virologe weiß, wird jede im Patienten nachgewiesene virale Genomkopie Indikator einer Infektion sein, sobald eine bestimmte Schwelle (also Anzahl nachgewiesener Genomkopien) überschritten ist.
This argument is completely mistaken.
I: No test at all can discriminate between complete and incomplete / fragmented viral genomes.
II: The ability to such discrimination is unnecessary because each genome copy - no matter if intact or damaged - originates from a cell infected with an intact virus.

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.

Zunächst einmal verwechseln die Autoren des Borger-Textes die Begriffe: Die ***Annealing***-Temperatur ist für beide gleich (das geht auch gar nicht anders!). Was sich unterscheidet, sind die ***Schmelz***temperaturen der beiden Primer. In der Tat sollten diese beiden Werte möglichst ähnlich sein. Aber jeder, der Erfahrung mit PCR-Systemen hat, weiß, dass die Gegebenheiten der vorliegenden Sequenzen das Machbare diktieren - größere Abweichungen von den optimalen Werten sind manchmal nicht vermeidbar! Auch aus diesem Grunde führt kein Weg am gründlichen Austesten und Optimieren der PCR-Bedingungen vorbei, was Drosten und Koautoren getan haben.
First, the authors confuse annealing temperature (which for both primers necessarily is identical) with melting temperature. Indeed, melting temperatures should be as similar as possible. But again: Everyone who has experience in PCR design knows that reality - i.e. the sequence context - dictates what is feasible. Greater deviations of melting temperatures sometimes are inevitable. This is one more reason for exhaustive testing of a diagnostic qPCR system which has been done by Corman et al.

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.

Wieder einmal verwechseln Borger et al. die Begriffe: Den Ct-Wert kann man gar nicht angeben, denn er wird gemessen. Gemeint ist wohl der Schwellenwert, der anzugeben sei, aber auch das ist unsinnig, weil diese Schwelle vom Detektionssystem (u.a. also auch dem Gerät) abhängt. Borger et al. scheinen nicht zu wissen, wie man eine qPCR durchführt: Parallel zu den Proben lässt man eine Kalibrierreihe "mitlaufen": Zusammen mit den Proben prozessiert man also eine Verdünnungsreihe, eine Serie von Proben mit bekannter Virus-Menge; erst der Vergleich mit den Ct-Werten dieser Reihe erbringt das Ergebnis.
Again Borger et al. mix up technical terms. You never can predefine a Ct value, since Ct is measured during each qPCR run for each sample. Probably the threshold is meant instead. But this also does not make any sense, because the threshold is dependent from the detection system and the qPCR machine used. Obviously, Borger et al. have no idea how to perform a qPCR test: Alongside with the samples, a dilution series is processed, serving as a standard to compare the samples with.

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.

Selbstverständlich wurden die PCR-Fragmente zu Beginn der Entwicklungsarbeiten gelelektrophoretisch analysiert! Das macht man ***immer*** so, aber es bedarf hier keiner Erwähnung: Die Spezifität bei einer solchen qPCR ist hoch wegen der drei voneinander unabhängigen Oligonukleotide (vgl. Punkt 1). Außerdem beschreibt das Corman-Drosten-Paper die Validierung in aller Ausführlichkeit.
Of course PCR fragments have been analysed by gel electrophoresis in the beginning! This is standard procedure! However, it needs not to be mentioned because specificity testing is more important. Moreover, the three independent oligonucleotides employed for TaqMan qPCR guarantee for high specificity. And the Drosten-Corma paper thoroughly documents validation of the qPCR system.

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.

Das Gegenteil ist wahr - das Corman-Drosten-Paper benennt mehrere Positiv- und Negativkontrollen und beschreibt ausführlich die Validierung der Test-Spezifität. Die konkrete Auswahl und "Konfektionierung" der Kontrollen obliegt dem Anwender bei Etablierung des qPCR-Systems, wenn er seine SOP definiert (vgl. Punkt 8). So ist das übliche Vorgehen.
The opposite is true. The Corman-Drosten paper describes some positive and negative controls as well as thorough validation. Precise setup of the test - including controls - is up to each individual user. And the user also has to define his own SOP.

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.

Die Behauptungen sind zur einen Hälfte unwahr, zur anderen Hälfte gehen sie am Thema vorbei: Tatsächlich benennt das Corman-Drosten-Paper alle relevanten Parameter: Oligonukleotid-Sequenzen und -konzentrationen, Salzbedingungen und dNTP-Konzentrationen, PCR-Zyklusbedingungen. SOPs sind hingegen auch (und in erheblichem Maße) abhängig von den örtlichen Gegebenheiten. Daher muss jeder diagnostische Test in jedem Labor erneut etabliert werden, und dazu gehört auch die Abfassung einer SOP. Auch hier zeigt sich, dass den Autoren des Borger-Testes jedwede Erfahrung mit diagnostischen Testsystemen fehlt.
Half of the claim is untrue, the other half is irrelevant. Indeed, the Corma-Drosten paper specifies all relevant information: Primer and probe sequences, concentrations, PCR conditions. On the other hand, formulation of the SOP is up to the user and not to the publisher of a test.

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.

In der Tat dauert ein Begutachtungsprozess mehrere Tage oder sogar Wochen, allerdings ist das Journal *Eurosurveillance* für seine Schnelligkeit bekannt. Und wenn

eine Publikation ansteht, bei der sich Autoren und Journal über die Dringlichkeit einig sind, dann wird manchmal ein beschleunigtes Verfahren durchgeführt: Entweder es wird dafür gesorgt, dass die Gutachter an Tag und Stunde der Einreichung bereit stehen. Oder die Begutachtung der Publikation erfolgt in Teilen - es wird laufend alles begutachtet, was eingereicht wird. Und in diesem Fall ist das in der Publikation vermerkte Datum der Einreichung der Tag, an dem das letzte Dokument eingereicht wurde - z.B. Material, das die Gutachter noch nachgefordert haben. In solchem Fall kann der vermerkte Tag der Einreichung auch gleichzeitig der Tag sein, an dem die Publikation akzeptiert wurde. Übrigens: In diesem speziellen Fall stand den Gutachtern das Corman-Drosten-Paper bereits eine Woche vor der offiziellen Einreichung über einen sog. Preprint-Server zur Verfügung...
Indeed, peer reviews usually take days or even weeks - *Eurosurveillance*, however, is known for high speed. Sometimes - especially when authors and journal agree that publication is urgent - arrangements are made: Either reviewers may stand by the very hour the paper is filed. Or the paper is filed and reviewed bit by bit. In such a case the noted day of submission is the day when the last part or the last data were filed. This may well be the day of approval. By the way - in the case of the Corman-Drosten publication, the paper was available to reviewers one week before on a preprint server...

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.

Ein Interessenskonflikt ergäbe sich dann - und ***nur*** dann - wenn benannte Koautoren einen wie auch immer gearteten Vorteil von ihrer Publikation hätten, z. B. durch Verkauf von Kits und Reagenzien oder durch Gewinn von Marktanteilen etc. Das in der Corman-Drosten- Publikation vorgestellte qPCR-System ist allerdings frei in seiner Verwendung. Jeder kann es anwenden und die Reagenzien dafür bei jedem beliebigen Lieferanten erstehen. Daher ist es schwer, diese Behauptungen anders zu interpretieren denn als böswillige Verleumdung.
Conflicts of interest only exist if the respective coauthors had an advantage from the publication, e.g. by marketing and distribution of kits or by gaining market share. However, the described qPCR system is freely available and applicable for everyone and reagents can be purchased from any distributor, Hence it is hard to interpret this claim of Borger et al. different than vicious slander.

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.

Sämtliche Vorwürfe im Borger-Text sind unbegründet. Die Argumentation ist unwissenschaftlich und zum größten Teil an den Haare herbei gezogen.
All points made by Borger et al. are unsubstantiated. Their line of agrumentation is pseudoscientific and totally wrong.

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1) **Dr. Peter Borger** (MSc, PhD), Molecular Genetics, W+W Research Associate, Lörrach, Germany

   Die Selbstbezeichnung ist eigentlich irreführend: "W+W" (*Wort und Wissen*) ist eine Kreationisten-Organisation, in der keine Laboratorien und Forschungsgruppen – und somit auch keine *Research Associates* – existieren.

   "W+W" (for "Word and Knowledge") is a creationist association. There are no laboratories and scientific research groups. And hence, there is also no "Research Associate".

2) **Rajesh Kumar Malhotra** (Artist Alias: **Bobby Rajesh Malhotra**), Former 3D Artist / Scientific Visualizations at CeMM - Center for Molecular Medicine of the Austrian Academy of Sciences (2019-2020), University for Applied Arts - Department for Digital Arts Vienna, Austria

Ein "3D-Künster“, ernsthaft??

A "3D-artist", seriously??

3) **Dr. Michael Yeadon** BSs(Hons) Biochem Tox U Surrey, PhD Pharmacology U Surrey. Managing Director, Yeadon Consulting Ltd, former Pfizer Chief Scientist, United Kingdom,

Früherer Pfizer-Mitarbeiter (Pharmakologe / Toxikologe / Manager) und Impfgegner / Verschwörungstheoretiker (siehe https://www.wodarg.com, https://unherd.com/2020/11/the-trouble-with-covid-denialism/, https://bylinetimes.com/2020/12/04/alt-right-pseudoscience-part-1-lockdown-sceptics/) - kein Molekularbiologe.

Former Pfizer employee (pharmacologist / toxicologist / manager) and anti-vaccationist / conspiracy theorist (https://www.wodarg.com/, https://unherd.com/2020/11/the-trouble-with-covid-denialism/, https://bylinetimes.com/2020/12/04/alt-right-pseudoscience-part-1-lockdown-sceptics/), not a molecular biologist

4) **Dr. Clare Craig** MA, (Cantab) BM, BCh (Oxon), FRCPath, United Kingdom

Eine Pathologin ist keine Molekularbiologin.

A pathologist is not a molecular biologist

5) **Kevin McKernan**, BS Emory University, Chief Scientific Officer, founder Medical Genomics, engineered the sequencing pipeline at WIBR/MIT for the Human Genome Project, Invented and developed the SOLiD sequencer, awarded patents related to PCR, DNA Isolation and Sequencing, USA

McKernan ist seit Jahrzehnten in Wissenschaftsmanagement und -organisation tätig; nun ist er führender Vertriebsmanager (https://www.medicinalgenomics.com/team/kevin-mckernan/), er hat seit vielen Jahren mit praktischer Wissenschaft nichts mehr zu tun.

Since decades McKernan is involved in science management and organisation, now he is chief sales officer and hence out of practical science since many years (https://www.medicinalgenomics.com/team/kevin-mckernan/)

6) **Prof. Dr. Klaus Steger**, Department of Urology, Pediatric Urology and Andrology, Molecular Andrology, Biomedical Research Center of the Justus Liebig University, Giessen, Germany

Ein Androloge und Urologe ist kein Molekularbiologe.

An andrologist / urologist is not a molecular biologist

7) **Dr. Paul McSheehy** (BSc, PhD), Biochemist & Industry Pharmacologist, Loerrach, Germany

Ein Biochemiker und Pharmakologe ist kein Molekularbiologe.

A biochemist / pharmacologist is not a molecular biologist

8) **Dr. Lidiya Angelova**, MSc in Biology, PhD in Microbiology, Former researcher at the National Institute of Allergy and Infectious Diseases (NIAID), Maryland, USA

Wir konnten keine wissenschaftlichen Publikationen von Dr. Angelova finden, die mit Corona oder mit diagnostischen PCR-Systemen zu tun hätten. Ferner scheint es so, als sei sie wissenschaftlich gar nicht mehr tätig.

We could not find any scientific publication of Dr. Angelova on Corona or on diagnostic PCR systems. Moreover it seems as if she has a different job now and is no more active in science.

9) **Dr. Fabio Franchi**, Former Dirigente Medico (M.D) in an Infectious Disease Ward, specialized in “Infectious Diseases” and “Hygiene and Preventive Medicine”, Società Scientifica per il Principio di Precauzione (SSPP), Italy

Ein Mediziner - Mikrobiologie, Hygiene, Infektiologie - ist kein Molekularbiologe. Keine seiner drei wissenschaftlichen Publikationen (https://www.dissensomedico.it/files/Curriculum-Vitae_CV_Fabio-Franchi.pdf) hat mit PCR-Systemen zu tun.

A physician - microbiology, hygiene, infectiology - is not a molecular biologist. None of his three scientific, peer-reviewed papers (https://www.dissensomedico.it/files/Curriculum-Vitae_CV_Fabio-Franchi.pdf) has to do with PCR-systems.

10) **Dr. med. Thomas Binder**, Internist and Cardiologist (FMH), Switzerland

Ein Internist und Kardiologe ist kein Molekularbiologe.

An internist / cardiologist is not a molecular biologist.

11) **Prof. Dr. med. Henrik Ullrich**, specialist Diagnostic Radiology, Chief Medical Doctor at the Center for Radiology of Collm Oschatz-Hospital, Germany

Ein Radiologe ist kein Molekularbiologe. Außerdem ist er der zweite Autor, welcher der pseudowissenschaftlichen Kreationisten-Organisation *Wort und Wissen* angehört – ja, er ist sogar deren Vorsitzender.

A radiologist is not a molecular biologist. besides this, he is the second author belonging to the pseudoscientific creationist organisation "Wort und Wissen" - he even is the chairman!

12) **Prof. Dr. Makoto Ohashi**, Professor emeritus, PhD in Microbiology and Immunology, Tokushima University, Japan

Prof. Ohashi war ein Spezialist für das Epstein-Barr-Virus sowie die dadurch ausgelösten Lymphome. Mit Corona oder diagnostischen PCRs hatte er nichts zu tun.
https://www.researchgate.net/scientific-contributions/Makoto-Ohashi-2004561900
https://scholar.google.com/citations?user=2HseyOsAAAAJ&hl=en

Prof. Ohashi was a specialist for Epstein-Barr-Virus as well as viral induced lymphoma. He never was involved in Corona research or in development of diagnostic PCR systems.
https://www.researchgate.net/scientific-contributions/Makoto-Ohashi-2004561900
https://scholar.google.com/citations?user=2HseyOsAAAAJ&hl=en

13) **Dr. Stefano Scoglio**, B.Sc. Ph.D., Microbiologist, Nutritionist, Italy

Ein Mikrobiologe und Ökotrophologe ist kein Molekularbiologe.

A microbiologist / nutritionist is not a molecular biologist

14) **Dr. Marjolein Doesburg-van Kleffens** (MSc, PhD), specialist in Laboratory Medicine (clinical chemistry), Maasziekenhuis Pantein, Beugen, the Netherlands

Ein Klinischer Chemiker ist kein Molekularbiologe.

A specialist in Laboratory Medicine is not a molecular biologist

15) **Dr. Dorothea Gilbert** (MSc, PhD), PhD Environmental Chemistry and Toxicology. DGI Consulting Services, Oslo, Norway Review Report - Corman-Drosten *et al.*, Eurosurveillance 2020

Eine Umweltchemikerin und Toxikologin ist keine Molekularbiologin.

An environmental chemist / toxicologist medicine is not a molecular biologist

16) **Dr. Rainer J. Klement**, PhD. Department of Radiation Oncology, Leopoldina Hospital

Schweinfurt, Germany

Ein Onkologe ist kein Molekularbiologe.

An oncologist is not a molecular biologist

17) **Dr. Ruth Schruefer**, PhD, human genetics/ immunology, Munich, Germany,

Promovierte 2006 in Immunologie. Sie scheint seitdem wissenschaftlich nicht mehr aktiv zu sein.

PhD in immunology 2006. She seems to be out of science since then.

18) **Dra. Berber W. Pieksma**, General Practitioner, The Netherlands

Allgemeinmediziner? Ernsthaft??

General Practitioner? Seriously??

19) **Dr. med. Jan Bonte** (GJ), Consultant Neurologist, the Netherlands

Eine Neurologe ist kein Molekularbiologe.

An neurologist is not a molecular biologist

20) **Dr. Bruno H. Dalle Carbonare** (Molecular biologist), IP specialist, BDC Basel, Switzerland

Dieser Herr hat seit Jahrzehnten nichts mehr mit Molekularbiologie zu tun, sondern nur noch mit Wissenschaftsmanagement und -organisation.

This person is out of practical science since decades. He is involved in science management and organisation instead.

21) **Dr. Kevin P. Corbett**, MSc Nursing (Kings College London) PhD (London South Bank) Social Sciences (Science & Technology Studies) London, England, UK

Ein Krankenpfleger und Sozialwissenschaftler, ernsthaft?

Nursing and Social Sciences, seriously?

22) **Prof. Dr. Ulrike Kämmerer**, specialist in Virology / Immunology / Human Biology / Cell Biology, University Hospital Würzburg, Germany

Prof. Kämmerers Schwerpunkte lt. ihrer Homepage an der Würzburger Frauenklinik, wo sie als wissenschaftliche Mitarbeiterin geführt wird (https://www.ukw.de/frauenklinik/team/detail/name/kaemmerer-ulrike/), seien Humanbiologie, Virologie, Immunologie, Zellbiologie. Unter "Forschung" findet man dann allerdings keine Virologie.
In Verbindung mit ihrer Person finden sich z. B. populärwissenschaftlicher Bücher über Krebs und Ernährung und in der wissenschaftlichen Literatur eine Vielzahl von Publikationen in Immunologie, Tumor- und Reproduktionsbiologie. Es gibt nur ein virologisches Paper mit ihr (als Koautorin) über Enteroviren aus dem Jahr 1998 und eines über PCR-Detektion von Picornaviren aus 1995.
Im Dezember 2020 gab es einen kurzen E-Mail-Wechsel zwischen Fr. Kämmerer und mir. Am 28.12.2020, 18:52 fragte ich sie nach ihren eigenen Erfahrungen mit qPCR-Systemen. Diese Frage wollte sie nicht beantworten.
Kurz: Man kann sie weder als Virologin noch als PCR-kundig bezeichnen. Allerdings macht sie als Verschwörungstheoretikerin von sich reden, so z. B. äußerte sie in einem Interview mit dem AfD-Organ "Die freie Welt" "PCR-Test ist wie Kaffeesatzlesen..."

Prof. Kämmerers expertise as given on her website at the Würzburg gynaecological hospital - where she is listed as research assistant (https://www.ukw.de/frauenklinik/team/detail/name/kaemmerer-ulrike/) - is human biology, virology, immunology and cell biology. However, the "research"-link on this site does not list virology. She is author of popular science books on cancer and nutrition.

Her scientific work comprises quite a few papers on immunology, cancer, and reproduction biology. There is only a virological paper on enteroviruses (with her as co-author) from 1998 and a paper on detection of picornavirus by PCR from 1995. There was a short e-mail exchange between her and me in December 2020. I asked her for her own experience and skills in qPCR. She refused to answer
In other words: She neither is a virologist nor an expert in PCR. However, she distributes conspiracy theory, e.g. in "Die freie Welt" - an internet-newspaper of the right-wing populist party AfD, where she said "PCR-test is like reading the tea leaves".

Zusammenfassung:

Die Autoren nehmen in Anspruch, das von Drosten und Koautoren publizierte Testsystem wissenschaftlich kritisieren zu können. Aber bereits die Umstände der Publikation sind nicht sachgerecht:

- Wenn man wissenschaftliche Kritik äußern will, sollte man sie **wissenschaftlich** publizieren, in einem wissenschaftlichen Journal mit Peer-Review (!!) und nicht in Form einer privaten Domain im Internet. Solange der Borger-Text also nicht von einem Journal geprüft und akzeptiert wurde (was aufgrund seiner Haar sträubenden Fehler nicht passieren wird), ist er nichts als private Meinungsäußerung einer Laiengruppe.
- Ebenfalls ist es mehr als fragwürdig, wenn hier ein Testsystem ohne jede experimentelle Überprüfung kritisiert wird - allein auf der Basis "theoretischer Argumentationen".
- Drittens: Die Autoren - einer von ihnen ein "3D-Künstler" - verfügen über keinerlei Expertise auf dem Gebiet diagnostischer / analytischer PCR, was man den kritisierten Punkten auch deutlich anmerkt. Nur ein einziger - Prof. Ohashi - ist Virologe, jedoch ohne Erfahrung in Corona-Forschung. Auf dieser Basis eine Peer-Review-Publikation einer renommierten, international anerkannten Gruppe zu attackieren, ist einfach nur vermessen.
- Viertens: Das von Drosten & Kollegen entwickelte qPCR-Testsystem wird seit Monaten angewendet. Es wäre längst aufgefallen und (wissenschaftlich!) kritisiert worden, wenn es auch nur ansatzweise die behaupteten Schwächen hätte. Ganz im Gegenteil: Etliche Gruppen haben die publizierten Tests verglichen und keine schweren Mängel im Charité-Test gefunden (z.B. Afzal 22020, Matheeussen et al. 2020, Nalla et al. 2020, Vogels et al. 2020 - lediglich der RdRp-Assay hat eine geringere Sensitivität als Vergleichstests).
- Fünftens kann man problemlos aufzeigen, dass die vorgebrachten Kritikpunkte samt und sonders einer näheren Betrachtung nicht Stand halten. **Dabei finden sich sogar etliche Anfängerfehler von Seiten Borgers et al.** Die zu erkennen braucht man aber leider Sachkenntnis, über die ein Laie nicht verfügt. Und so macht diese "Publikation" die Runde unter Verschwörungstheoretikern, "Querdenkern", Impfgegnern und Kreationisten: *Audacter calumniare - semper aliquid haeret*...
- Letztlich gab ich den Autoren eine Woche Zeit, sich zu dieser Analyse zu äußern. Sie schwiegen.

Insgesamt kann man bei dieser Publikation bestenfalls von Pseudowissenschaft - wenn nicht von Verschwörungstheorie und Verleumdung - sprechen.

Summary

The authors claim to be able to refute the Drosten paper on scientific grounds.

- However, if you want to criticise a scientific paper, you should do this in the form of another scientific paper, published in a scientific journal with peer review (!) and not in the form of a private www-domain. This fact alone already renders this "peer review" highly dubious. Hence, as long as the text by Borger et al. has not been checked and accepted by a scientific journal (which will not happen due to its blatant errors) it is nothing than expression of opinion of some laypeople.
- Second, the fact that the authors did not perform a single experiment but argue alone on basis of "theoretical considerations" is more than questionable.
- Third, the authors have no expertise in analytic / diagnostic PCR systems. There is only a single virologist among them - Prof. Ohashi. And he has no experience in corona research. The ignorance of the authors can easily be recognised by the flawed critique they utter. **Some of the mistakes they make even are beginner's mistakes**. It is nothing but imprudence to criticise on such weak grounds a peer-reviewed paper from an internationally well-respected scientific group.
- Fourth, the respective qPCR-system has now been used worldwide since several months. If it was faulty, if it had any of the claimed impairments it would have been criticised long ago. In contrary - meanwhile there have quite a few comparisons of existing PCR assays been published (z. B. Afzal 22020, Matheeussen et al. 2020, Nalla et al. 2020, Vogels et al. 2020). No one has found sever drawbacks in the Corman-Drosten assay (just a less sensitive RdRp-assay in comparison to others, that was all).
- Fifth, it can easily be shown that all critical points submitted by the authors implode under closer inspection. However, quite a bit technical knowledge - not available for laypeople - is necessary to see through this flawed argumentation. And so, this "paper" already has become famous among conspiracy theory proponents, anti-vaccationists and creationists: *Audacter calumniare - semper aliquid haeret*...
- Last, Borger et al. were given the chance to comment on my arguments and to defend themselves. They kept silent.

In all, this "peer review" is at best pseudoscience – if not conspiracy theory and slander.

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