Review report Corman-Drosten et al. Eurosurveillance 2020

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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.

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ABSTRACT

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

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

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

Considering the scientific and methodological blemishes presented here, we are confident that the editorial board of Eurosurveillance has no other choice but to retract the publication.

CONCISE REVIEW REPORT

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

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

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

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

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

perform these experiments and provide the crucial data.

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

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

> ***"the establishment and validation of a diagnostic workflow for 2019-nCoV screening and specific confirmation, designed in absence of available virus isolates or original patient specimens. Design and validation were enabled by the close genetic relatedness to the 2003 SARS-CoV, and aided by the use of synthetic nucleic acid technology."***

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

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

1. The primers and probes:

a) the concentration of primers and probes must be of optimal range (100-200 nM)
b) must be specific to the target-gene you want to amplify
c) must have an optimal percentage of GC content relative to the total nitrogenous bases (minimum 40%, maximum 60%)
d) for virus diagnostics at least 3 primer pairs must detect 3 viral genes (preferably as far apart as possible in the viral genome)

2. The temperature at which all reactions take place:

a) DNA melting temperature (>92°)
b) DNA amplification temperature (TaqPol specific)
c) Tm; the annealing temperature (the temperature at which the primers and probes reach the target binding/detachment, not to exceed 2℃ per primer pair). Tm heavily depends on GC content of the primers

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

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

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

5. Positive and negative controls should be specified to

confirm/refute specific virus detection

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

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

MINOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

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

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

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

MAJOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

A) BACKGROUND

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

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

As an aim the authors declared to develop and deploy robust diagnostic methodology for use in public health laboratory settings without having virus material available. Further, they acknowledge that "The present study demonstrates the enormous response capacity achieved through coordination of academic and public laboratories in national and European research networks."

B) METHODS AND RESULTS

1. Primer & Probe Design

1a) Erroneous primer concentrations

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

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

Table1: Primers and probes (adapted from Corman-Drosten paper; erroneous primer

concentrations are highlighted)

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

[a] W is A/T; R is G/A; M is A/C; S is G/C. FAM: 6-carboxyfluorescein; BBQ: blackberry quencher.
[b] Optimised concentrations are given in nanomol per litre (nM) based on the final reaction mix, e.g. 1.5 µL of a 10 µM primer stock solution per 25 µL total reaction volume yields a final concentration of 600 nM as indicated in the table.

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

To obtain reproducible and comparable results, it is essential to distinctively define the primer pairs. In the Corman-Drosten paper we observed six unspecified positions, indicated by the letters R, W, M and S (Table 2). The letter W means that at this position there can be either an A or a T; R signifies there can be either a G or an A; M indicates that the position may either be an A or a C; the letter S indicates there can be either a G or a C on this position.

This high number of variants not only is unusual, but it also is highly confusing for laboratories. These six unspecified positions could easily result in the design of several different alternative primer sequences which do not relate to SARS-CoV-2 (2 distinct RdRp_SARSr_F primers + 8 distinct RdRp_SARS_P1 probes + 4 distinct RdRp_SARSr_R). **The design variations will inevitably lead to results that are not even SARS CoV-2 related. Therefore, the confusing unspecific description in the Corman-Drosten paper is not suitable as a Standard Operational Protocol. These unspecified positions should have been designed unequivocally.**

These wobbly sequences have already created a source of concern in the field and resulted in a Letter to the Editor authored by Pillonel et al. [8] regarding

blatant errors in the described sequences. These errors are self-evident in the Corman et al. supplement as well.

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

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

[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.

The WHO-protocol (Figure 1), which directly derives from the Corman-Drosten paper, concludes that in order to confirm the presence of SARS-CoV-2, two control genes (the E-and the RdRp-genes) must be identified in the assay. It should be noted, that the RdPd-gene has one uncertain position ("wobbly") in the forward-primer (R=G/A), two uncertain positions in the reverse-primer (R=G/A; S=G/C) and it has three uncertain positions in the RdRp-probe (W=A/T; R=G/A; M=A/C). So, two different forward primers, four different reverse primers, and eight distinct probes can be synthesized for the RdPd-gene. Together, there are 64 possible combinations of primers and probes!

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

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

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

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

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

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

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

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

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

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

1d) Detection of viral genes

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

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

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

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

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

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

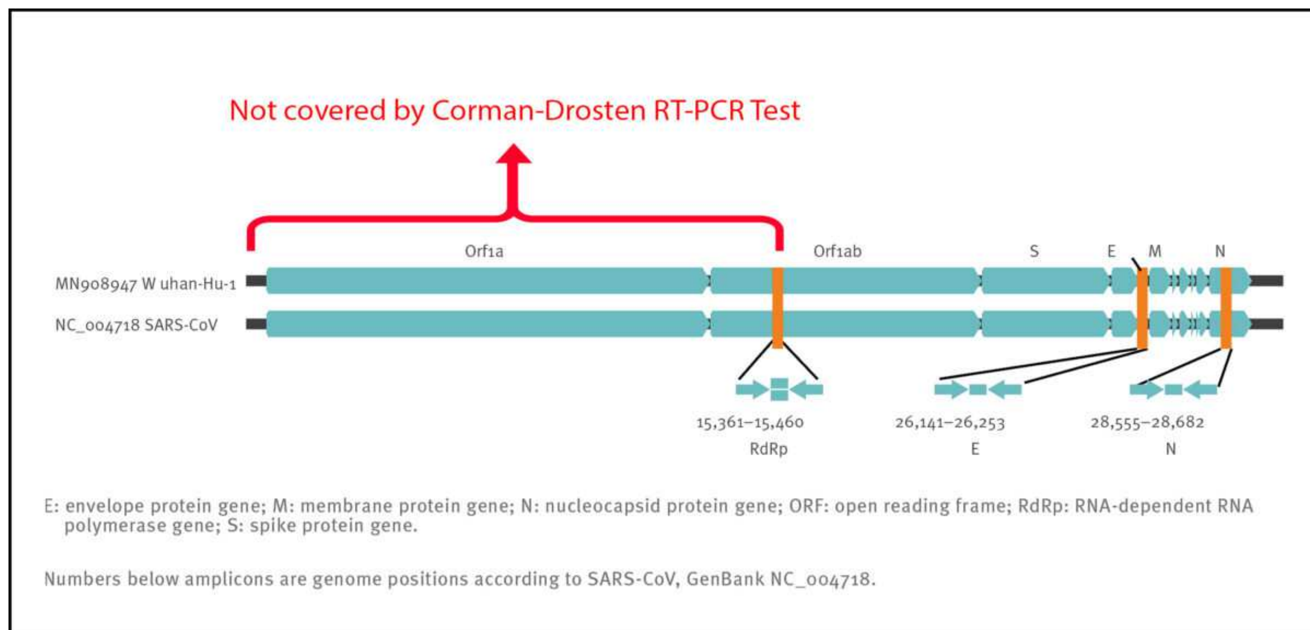
Kim et al. demonstrate a highly variable 3' expression of subgenomic RNA in Sars-CoV-2 [23]. These RNAs are actively monitored as signatures for asymptomatic and non-infectious patients [10]. It is highly questionable to screen a population of asymptomatic people with qPCR primers that have 6 base pairs primer-dimer on the 3 prime end of a primer (Figure 3).
Apparently the WHO recommends these primers. We tested all the wobble derivatives from the Corman-Drosten paper with Thermofisher's primer dimer web tool [11]. The RdRp forward primer has 6bp 3prime homology with Sarbeco E Reverse. At high primer concentrations this is enough to create inaccuracies.

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

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

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

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



E: envelope protein gene; M: membrane protein gene; N: nucleocapsid protein gene; ORF: open reading frame; RdRp: RNA-dependent RNA polymerase gene; S: spike protein gene.

Numbers below amplicons are genome positions according to SARS-CoV, GenBank NC_004718.

```
Cross Primer Dimers:

Corman_RdRp_SARs_F1 with Corman_E_Sarbeco_R
Corman_RdRp_SARs_F1
5-gtgaaatggtcatgtgtggcgg->
             ||||||
        <-acacacgcatgacgacgttata-5

Corman_RdRp_SARs_F2 with Corman_E_Sarbeco_R
Corman_RdRp_SARs_F2
5-gtgagatggtcatgtgtggcgg->
             ||||||
        <-acacacgcatgacgacgttata-5
```

>Corman_N_Sarbeco_F

CACATTGGCACCCGCAATC

Pantoea agglomerans strain ASB05 chromosome, complete genome

Sequence ID: CP046722.1 Length: 4022781 Number of Matches: 2

Range 1: 2326019 to 2326037 GenBank Graphics ▼ Next Match

Score	Expect	Identities	Gaps	Strand
38.2 bits(19)	2.2	19/19(100%)	0/19(0%)	Plus/Plus

```
Query  1        CACATTGGCACCCGCAATC  19
                |||||||||||||||||||
Sbjct  2326019  CACATTGGCACCCGCAATC  2326037
```

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.

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

We used the freely available primer design software Primer-BLAST [12, 25] to evaluable the best-practise values for all primers used in the Corman-Drosten paper (Table 3). We attempted to find a Tm-value of 60° C, while similarly

seeking the highest possible GC%-value for all primers. A maximal Tm difference of 2° C within primer pairs was considered acceptable. Testing the primer pairs specified in the Corman-Drosten paper, we observed a difference of 10° C with respect to the annealing temperature Tm for primer pair1 (RdRp_SARSr_F and RdRp_SARSr_R). **This is a very serious error and makes the protocol useless as a specific diagnostic tool.**

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

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

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

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

GC% 59,09 TM 63,74°

Difference of almost 10°

GC% 28,00 TM 53,56°

GC% 34,62 TM 58,29°

GC% 45,45% TM 60,93°

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

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

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

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

3. The number of amplification cycles

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

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

Citing Jaafar et al. 2020 [3]: “At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive.” **In other words, there was no**

successful virus isolation of SARS-CoV-2 at those high Ct values.

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

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

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

3. Discrimatory assay

RdRp assay:

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

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

Cycler:

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

4. Biomolecular validations

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

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

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

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

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

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

All individuals testing positive with the RT-PCR test, as described in the Corman-Drosten paper, are assumed to be positive for SARS-CoV-2 infections. There are three severe flaws in their assumption. First, a positive test for the RNA molecules described in the Corman-Drosten paper cannot be equated to "infection with a virus". A positive RT-PCR test merely indicates the presence of viral RNA molecules. As demonstrated under point 1d (above), **the Corman-Drosten test was not designed to detect the full-length virus, but only a fragment of the virus. We already concluded that this classifies the test as unsuitable as a diagnostic test**
for SARS-virus infections.

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

Third, the Corman-Drosten paper states:

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

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

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

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

Furthermore, the absence of the HE gene in both SARS-CoV1 and SARS-CoV-2 makes this gene the ideal negative control to exclude other coronaviruses. The Corman-Drosten paper does not contain this negative control, nor does it contain any other negative controls. **The PCR test in the Corman-Drosten paper therefore contains neither a unique positive control nor a negative control to exclude the presence of other coronaviruses. This is another major design flaw which classifies the test as unsuitable for diagnosis.**

6. Standard Operational Procedure (SOP) is not available

There should be a Standard Operational Procedure (SOP) available, which unequivocally specifies the above parameters, so that all laboratories are able to set up the identical same test conditions. To have a validated universal SOP is essential, because it facilitates data comparison within and between countries. **It is very important to specify all primer parameters unequivocally. We note that this has not been done.** Further, the Ct value to indicate when a sample should be considered positive or negative is not specified. It is also not specified when a sample is considered infected with SARS-CoV viruses. As shown above, the test cannot discern between virus and virus fragments, so the Ct value

indicating positivity is crucially important. This Ct value should have been specified in the Standard Operational Procedure (SOP) and put on-line so that all laboratories carrying out this test have exactly the same boundary conditions. It points to flawed science that such an SOP does not exist. The laboratories are thus free to conduct the test as they consider appropriate, resulting in an enormous amount of variation. Laboratories all over Europe are left with a multitude of questions; which primers to order? which nucleotides to fill in the undefined places? which Tm value to choose? How many PCR cycles to run? At what Ct value is the sample positive? And when is it negative? And how many genes to test? Should all genes be tested, or just the E and RpRd gene as shown in Table 2 of the Corman-Drosten paper? Should the N gene be tested as well? And what is their negative control? What is their positive control?

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

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

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

> ***"In four individual test reactions, weak initial reactivity was seen however they were negative upon retesting with***

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

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

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

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

Before formal publication in a scholarly journal, scientific and medical articles are traditionally certified by "peer review." In this process, the journal's editors take advice from various experts ("referees") who have assessed the paper and may identify weaknesses in its assumptions, methods, and conclusions.

Typically a journal will only publish an article once the editors are satisfied that the authors have addressed referees' concerns and that the data presented supports the conclusions drawn in the paper." This process is as well described for Eurosurveillance [16].

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

Normally, peer review is a time-consuming process since at least two experts from the field have to critically read and comment on the submitted paper. In our opinion, this paper was not peer-reviewed. Twenty-four hours are simply not enough to carry out a thorough peer review. Our conclusion is supported by the fact that a tremendous number of very serious design flaws were found by us, which make the PCR test completely unsuitable as a diagnostic tool to identify the SARS-CoV-2 virus. Any molecular biologist familiar with RT-PCR design would have easily observed the grave errors present in the Corman-Drosten paper before the actual review process. We asked Eurosurveillance on October 26th 2020 to send us a copy of the peer review report. To date, we have not received this report and in a letter dated November 18th 2020, the ECDC as host for Eurosurveillance declined to provide access without providing substantial scientific reasons for their decision. On the contrary, they write that "disclosure would undermine the purpose of scientific investigations." [24].

9. Authors as the editors

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

because the authors were also part of the editorial board at Eurosurveillance. This practice is categorized as compromising scientific integrity.

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

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

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

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

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

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

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

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

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

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

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

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

CONCLUSION

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

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

REFERENCES

[1] Corman Victor M, Landt Olfert, Kaiser Marco, Molenkamp Richard, Meijer Adam, Chu Daniel KW, Bleicker Tobias, Brünink Sebastian, Schneider Julia, Schmidt Marie Luisa, Mulders Daphne GJC, Haagmans Bart L, van der Veer Bas, van den Brink Sharon, Wijsman Lisa, Goderski Gabriel, Romette Jean-Louis, Ellis Joanna, Zambon Maria, Peiris Malik, Goossens Herman, Reusken Chantal, Koopmans Marion PG, Drosten Christian. Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR. Euro Surveill. 2020;25(3):pii=2000045. https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045

[2] Email communication between Dr. Peter Borger & Dr. Adam Meijer: Supplementary Material

[3] Jafaar et al., Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates. https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1491/5912603

[4] BBC, January 21st 2020: https://www.bbc.com/news/world-asia-china-51185836;
Archive: https://archive.is/0qRmZ

[5] Google Analytics – COVID19-deaths worldwide: https://bit.ly/3fndemJ
Archive: https://archive.is/PpqEE

[6] Laboratory testing for COVID-19 Emergency Response Technical Centre, NIVD under China CDC March 15th, 2020: http://www.chinacdc.cn/en/COVID19/202003/P020200323390321297894.pdf

[7] Real-Time PCR Handbook Life Technologies: https://www.thermofisher.com/content/dam/LifeTech/global/Forms/PDF/real-time-pcr-

handbook.pdf

Nolan T, Huggett J, Sanchez E.Good practice guide for the application of quantitative PCR (qPCR) First Edition 2013

[8] Trestan Pillonel et al, Letter to the editor: SARS-CoV-2 detection by real-time RT-PCR: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7268274/

[9] Kurkela, Satu, and David WG Brown. "Molecular-diagnostic techniques." Medicine 38.10 (2009): 535-540.

[10] Wolfel et al., Virological assessment of hospitalized patients with COVID-2019 https://www.nature.com/articles/s41586-020-2196-x

[11] Thermofischer Primer Dimer Web Tool: https://www.thermofisher.com/us/en/home/brands/thermo-scientific/molecular-biology/molecular-biology-learning-center/molecular-biology-resource-library/thermo-scientific-web-tools/multiple-primer-analyzer.html

Supplementary Material

[12] Primer-BLAST, NCBI – National Center for Biotechnology Information: https://www.ncbi.nlm.nih.gov/tools/primer-blast/

[13] Marra MA, Steven JMJ, Caroline RA, Robert AH, Angela BW et al. (2003) Science. The Genome sequence of the SARS-associated coronavirus. Science 300(5624): 1399-1404.

[14] Severe acute respiratory syndrome coronavirus 2 isolate Wuhan-Hu-1, complete genome: https://www.ncbi.nlm.nih.gov/nuccore/MN908947

[15] Borger P. A SARS-like Coronavirus was expected but nothing was done to be prepared. Am J Biomed Sci Res 2020. https://biomedgrid.com/pdf/AJBSR.MS.ID.001312.pdf
https://www.researchgate.net/publication/341120750_A_SARS-like_Coronavirus_was_Expected_but_nothing_was_done_to_be_Prepared;
Archive: https://archive.is/i76Hu

[16] Eurosurveillance paper evaluation / review process: https://www.eurosurveillance.org/evaluation

[17] Official recommendation of the Corman-Drosten protocol & manuscript by the WHO,published

on January 13th 2020 as version 1.0 of the document:
https://www.who.int/docs/default-source/coronaviruse/wuhan-virus-assay-v1991527e5122341d99287a1b17c111902.pdf; archive: https://bit.ly/3m3jXVH

[18] Official WHO-recommendation for the Corman / Drosten RT-qPCR-protocol, which directly derives from the Eurosurveillance-publication, document-version 2-1, published on 17th January 2020: https://www.who.int/docs/default-source/coronaviruse/protocol-v2-1.pdf?sfvrsn=a9ef618c_2

[19] Eurosurveillance Editorial Board, 2020: https://www.eurosurveillance.org/upload/site-assets/imgs/2020-09-Editorial%20Board%20PDF.pdf;
Archive: https://bit.ly/2TqXBjX

[20] Instructions For Use LightMix SarbecoV E-gene plus EAV Control, TIB-Molbiol & Roche Molecular Solutions, January 11th 2020: https://www.roche-as.es/lm_pdf/MDx_40-0776_96_Sarbeco-E-gene_V200204_09164154001 (1).pdf
Archive, timestamp – January 11th 2020: https://archive.is/Vulo5;
Archive: https://bit.ly/3fm9bXH

[21] Christian Drosten & Victor Corman, responsible for viral diagnostics at Labor Berlin:
https://www.laborberlin.com/fachbereiche/virologie/
Archive: https://archive.is/CDEUG

[22] Tom Jefferson, Elizabeth Spencer, Jon Brassey, Carl Heneghan Viral cultures for COVID-19 infectivity assessment. Systematic review. Systematic review doi:
https://doi.org/10.1101/2020.08.04.20167932 https://www.medrxiv.org/content/10.1101/2020.08.04.20167932v4

[23] Kim et al.,The Architecture of SARS-CoV-2 Transcriptome:
https://www.sciencedirect.com/science/article/pii/S0092867420304062

[24] ECDC reply to Dr. Peter Borger, 18th November 2020:
Supplementary Material

[25] Prof. Dr. Ulrike Kämmerer & team, survey & Primer-BLAST table:
Supplementary Material

Additional literature:

Description RT-PCR RKI Germany, on page 10 of this link:
https://www.rki.de/DE/Content/Gesundheitsmonitoring/Gesundheitsberichterstattung/GBE DownloadsJ/JoHM_S5_2020_Studienprotokoll_CORONA_MONITORING_lokal.pdf?__blob=publicationFile

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MY: Conducted the analyses and research.

KMcK: Conducted the analyses and research, conceptualized the manuscript.

KS: Conducted the analyses and research.

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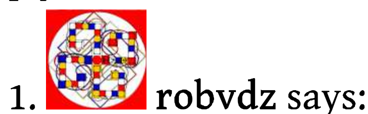
next »

Comments

1. **Sebe Vpgel** says:
   November 29, 2020 at 11:36 pm

   Thanks for your excellent work!

   Reply

   1.  **robvdz** says:
      November 30, 2020 at 6:58 pm

      Beside all of this, I won't let somebody that is not medically schooled

fidle around in my nose cavities for what ever reason.

Reply

1. **Bayaba** says:
December 1, 2020 at 1:44 am

I won't let someone who is medically trained do that. That includes my own brother, who is an MD and who is the president and CEO of the family practice he runs.

Reply

2. **Dr. med. dent. Klaus Wilhelm Rocholl** says:
November 30, 2020 at 12:43 am

Congratulation – and my deepest and most sincere admiration for your impressive work.
I hope you maybe literally helped to save the world!

Reply

1. **Wim Sturm** says:
November 30, 2020 at 5:25 pm

Thank you for your great work!

Facts outweigh fiction and open people's eyes to this ridiculous fictional reality that has been created in the world based on the Corman Drosten paper.

Thank you again for distinguishing fiction from reality with you retraction paper.

Reply

3. **J.** says:
   November 30, 2020 at 12:50 am

   Great job! Heroes of the universe!!!

   Reply
4. **LIVIANA*** says:
   November 30, 2020 at 2:26 am

   Thank you
   Dankuwel
   Obrigado
   Merci
   Vielen dank
   Und viel Erfolg

   Reply
5. **DUC** says:
   November 30, 2020 at 2:37 am

   Thanks for publishing what I have been saying in essence all along (but sure not in such detail). Lets hope there are consequences which are in balance with the damage done to the world population.

   M.sc., D.sc., former researcher in molecular genetics, HIV, immunology, among others 6 y at NIH(USA)

   Reply
6. **D. Krüger** says:
   November 30, 2020 at 3:05 am

   Ein wundervoller Hoffnungsschimmer am Horizont der dem ganzen Irrsinn

hoffentlich ein baldiges Ende setzt bevor noch mehr unnötiges Leid und Elend verursacht wird.

Herzlichen Dank, für Ihre wissenschaftliche Integrität die einigen Ihrer Kollegen offenbar abhanden gekommen ist !

Reply

7. **Hoijtink** says:
   November 30, 2020 at 3:20 am

   Good to see that at least some scientists still use common sense and brains. In my book you are heroes. Sadly it remains to be seen what politicians will do next, they have their own (hidden?) agenda.

   Thank you all ....

   Reply

8. **Solveig Warren** says:
   November 30, 2020 at 4:26 am

   Thank you for telling the truth in such a professional manner and using science appropriately! You are true heroes of our Universe! It is a tough job to to stand up for honesty with the Media having a one track mind!

   Reply

9. **Marlee Ponich** says:
   November 30, 2020 at 4:48 am

   Much love and gratitude!

   Reply

10. **Autoglas** says:
    November 30, 2020 at 6:05 am

Thank you for all … I hope the best

Reply

11. **Fred K.** says:
November 30, 2020 at 6:26 am

Many thanks for the extremely good and bitterly necessary work! I hope that this work can make a fundamental contribution to finally putting a stop to the madness. If the faulty paper is not voluntarily withdrawn by the authors, I very much hope that there is a way to force this through the courts with the help of dedicated lawyers. Thank you for your extraordinary commitment!

Reply

12. **Christoph Schmitz (Univ.Prof. Dr.med.)** says:
November 30, 2020 at 6:41 am

I cannot comment on PCR issues; this is not my field of expertise (I am a neuroanatomist at LMU Munich/Germany with approximately 200 papers listed in PubMed). I would just like to comment on the “points of major concern” #8 and #9 outlined above:

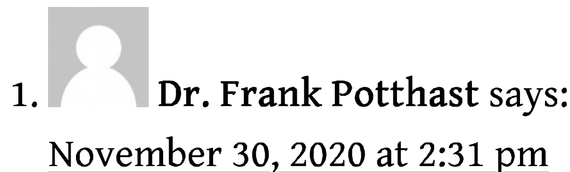
#8: it is indeed feasible (and not unusual) to perform a scientifically sound peer-review of a manuscript within a few hours after having obtained the request by a journal, particularly if you are an expert in the field. The fact that the manuscript that is discussed here was accepted for publication one day after submission does not mean that it was not peer-reviewed.

#9: every serious academic journal has an internal policy that manuscripts submitted by a member (or members) of the editorial board are handled and reviewed by other members of the editorial board of the journal. The fact that Christian Drosten has served as corresponding author of the manuscript described here does not imply that “scientific integrity was compromised”. In particular, this fact may not support the “suspicions that

the paper was not peer-reviewed".

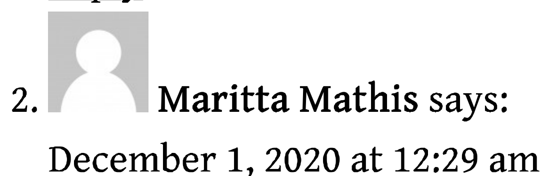
In summary, I warmly recommend to separate the "points of major concern" #8 and #9 outlined above from the other points of major concern in order to place this important discussion on a more objective footing.

Reply

1.  **Dr. Frank Potthast** says:
   November 30, 2020 at 2:31 pm

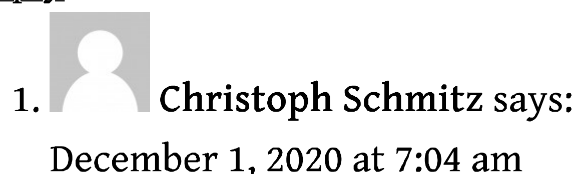
   In my opinion, you cannot separate the issues; if the editorial board has commmon sense, that this publication should be accepted for publication within a few hours, the scientific quality must be double-checked if you don´t want to risk accuses of wangle.
   The mistakes concerning good laboratory practice are so obvious, that I cannot believe, that it wouldn´t have been noticed by any of the experts.

   Reply

2.  **Maritta Mathis** says:
   December 1, 2020 at 12:29 am

   With all due respect, have you not actually recognized the dimensions and scope of this scandal, that you only criticize these two points, but are silent overall about the outrageously unscientific approach (where I live this is called fraud)?

   Reply

   1.  **Christoph Schmitz** says:
      December 1, 2020 at 7:04 am

      Please read my comment again. I simply cannot comment on PCR issues; this is not my field of expertise (I have never worked with

PRC). This must be done by others (which I highly welcome, no doubt). However, when reading all these comments here it looks that there are so many experts around that my non-expert opinion is really not necessary.

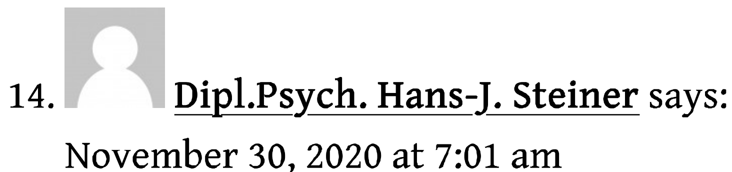
Reply

13. **Els van Veen** says:
November 30, 2020 at 6:42 am

Danke! Dankuwel! Thank you!

Ik ben een Nederlandse huisarts en hoop dat nu snel het krankzinnige testen (buiten de kliniek) kan worden gestaakt.
De lockdowns opgeheven.
De mondkapjes weggedaan.
De wetenschap in ere hersteld.
Het recht zal winnen van het onrecht en de leugen.

Reply

14.  **Dipl.Psych. Hans-J. Steiner** says:
November 30, 2020 at 7:01 am

Interesting work, however – after all tthese hoax-reports and "scientific" looking statements of people all over the wolrd, which came finally out, not being cited corectly or just abused for never done citations or just the person was "virtual", there will be much work to immunize that work from "Faktencheckers" and other discreditions. That will be even the more relevant work to be done urgently to make this paper an evicent part of public, scientific and political discussion!

Reply

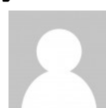
1. **Udo** says:

November 30, 2020 at 10:19 am

Dear Hans,

that is a very valid point. I wonder already since a long time about the “circular reasoning” in the so called “fact check”, as generally speaking they don’t make sense or the find “one specialst” who will explain it (putting a few minutes of effort in it).

Reply

 **Bobby** says:
November 30, 2020 at 2:47 pm

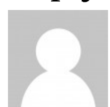
https://www.dailywire.com/news/candace-owens-challenges-fact-checker-and-wins

Reply

15. **Monika** says:
November 30, 2020 at 7:58 am

Thank You All for the great work! God bless you!

Reply

16.  **Dr .Andreas Gloge** says:
November 30, 2020 at 8:22 am

Man muß das Wahre immer wiederholen, weil auch der Irrtum um uns her immer wieder gepredigt wird, und zwar nicht von einzelnen, sondern von der Masse. In Zeitungen und Enzyklopädien, auf Schulen und Universitäten, überall ist der Irrtum oben auf, und es ist ihm wohl und behaglich, im Gefühl der Majorität, die auf seiner Seite ist.

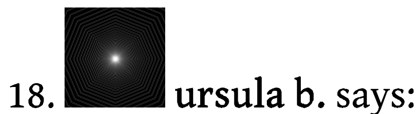
Johann Wolfgang von Goethe

Reply

17. **Brigid** says:
November 30, 2020 at 9:13 am

Thank you for this excellent piece of work which clarifies in detail and in a factual way what is felt by many. This is the proof. My hope is that this will enlighten those who need to know.

Reply

18.  **ursula b.** says:
November 30, 2020 at 9:54 am

The more one tries to opress truth the brighter it shines throughout the universe.
Thank you so much for your great work and dedication. With many small steps we will win the race 1nce and forever

Reply

19. **STRNTVRVLND** says:
November 30, 2020 at 9:56 am

Let's hope this effort will put a end to the disproportionate mesures. Please make this understandable for all by also presenting this information on micro-level instead of moleculare.

May a revolution be upon us

Reply

20. **B Anderson** says:
November 30, 2020 at 10:01 am

So, if the test to confirm you have covid-19 is useless...what virus is the

vaccine that is soon to be distributed going to prevent you from getting?

Reply

1. **Eva** says:
   November 30, 2020 at 7:10 pm

   You are right. And The Expert answers: "Good question, thank you. Next question, please…"

   Reply

   1. **human** says:
      November 30, 2020 at 10:00 pm

      Actually, we are no longer permitted to question anything.

      Because once questions are allowed, the answers will inevitably indict extremely powerful individuals and organizations of crimes against humanity.

      Reply

21. **Maria** says:
    November 30, 2020 at 10:26 am

    Thank you for doing this work and bringing real science to the table. Hoepfully this will end this epidemic of false positives which is causing so much human suffering. The cost of lockdowns: https://www.aier.org/article/cost-of-us-lockdowns-a-preliminary-report/

    Reply

22. **Robert Michel** says:
    November 30, 2020 at 10:32 am

Thank you,

If you have an hammer – every thing looks like a nail….
It becomes dangerious if political leader says "we will be only rescued, when we would have that vacination" and Media is following narrowminded.

We are spending Billions just on test-positiv cases on a RTPCR-test of one Corona-Virus,
instead looking on infektion problems in general.

Where are the programs to prevent infected people will not become hostpital patients?

Why not use unspecific inflammation marker CRP test to prevent infections in hospitals
and nursing homes? Every worker could be tested on CRP strikings before his shift, just
one drop blood, <5€ test and 5 minutes. There are many infection beside of Corona aviodable,
when health (also of the workes from doctor to the cleaning stuff) and not the duty rosta
and financial proffit would roule (have higest priority).
Influnenca, Streptokken, Pneumokokken, MSRA… all other infectes spread by doctors are
dangerious for their patients. In case of a CRP strike, more diagnostic should follow
before first contact with patients.
Over 200 doctors and medical workes had died in Italy in spring, not because the virus
is so agressive, major reason has been the working condition, to countious to work even
with illness.

Such test could help to stop the need to work full time with mask.

Why not having just a study about this, that the medical and care busines could do more
than washing hands and wearing masks.

A Chinese guidline how to deal with corona cases from March 2020 recomended to do
CRP tests in early stages it was translated and recomended by an German association of
eye clinics: http://www.vsdar.de/corona/
From March to May did I request action by authorities in NRW and Germany nd try to make
this public with small demoes in front of the German health ministery in Bonn, and in
the center of Bonn – see: http://www.corona-demo.de

IMHO a CRP could help to distinguish between persons with inhalated Corona-Gen and being
infected, too. But officials in Germany (RKI) count death with several negativ PCR-test
to the Corona-Death-counter: https://heise.de/-4973792.

I'm just an civil engineer, but I learnd as helper in 1994 in Goma and Bukavu (Kongo)
during the colera epedime from experienced developing professionls that it is important
to have an open exchance about challanges, ressources, ideas, and experiances.

So thank you again for your review, what do you think about CRP tests?
Robert Michel, Germany

Reply

23. **Ruud van Wees** says:
November 30, 2020 at 10:34 am

Thanks so much, all of you, the real scientists annex freedom fighters.
So many branches of science these days seem corrupted and sent into deadend streets by scientists bowing for the mammon or other kinds of pressure.
I dearly hope this is the beginning of the end of this politicized corona nightmare. If not, then we know for sure there is another dark agenda behind it.

Reply

24. **jb** says:
November 30, 2020 at 11:06 am

Remember, this pandemic has started with hiding the truth that dr Li Wenliang told. Please keep in mind his last words in his poem: "...Goodbye, my dear ones.
Farewell, Wuhan, my hometown.
Hopefully, after the disaster,
You'll remember someone once
Tried to let you know the truth as soon as possible.
Hopefully, after the disaster,
You'll learn what it means to be righteous...."

Reply

1. **human** says:
November 30, 2020 at 10:07 pm

What is your evidence that Li Wenliang story is factual?

Since when does the Communist Party of China apologize to a citizen? They made an exception for Li Wenliang.

"Hopefully, after the disaster"

Is this the "plague" that the good doctor is talking about?

"Tried to let you know the truth as soon as possible."

Dr. Li sounds like a fictional character out of PLA psyops units. His story was to sell the fiction of people dropping dead in Wuhan. (Remember those?) His warning about the "disaster" was fuel for the propaganda fire of the plague that is not a plague.

"You'll learn what it means to be righteous…."

That sounds ominous. Is that an oblique reference to coming re-education camps for plague deniers?

Reply

25. **Rehabilitation** says:
November 30, 2020 at 11:16 am

I was suggested this blog by my cousin. I am not sure whether this post is written by him as nobody else know such detailed about my problem. You are amazing! Thanks!

Reply

26. **frank** says:
November 30, 2020 at 11:20 am

Why you removed the reply's from willem engel, who is talking about a fungus.

Reply

Bobby says:
November 30, 2020 at 12:34 pm

There was a Bug in the comment-system and some comments vanished (2 or 3), this problem has been fixed.

Reply

27. **Mario Wolf** says:
November 30, 2020 at 11:47 am

Excellent. Hopefully this clarification will have the necessary impact

Reply

28. **Guy Verstraeten** says:
November 30, 2020 at 11:58 am

Eindelijk , en nu hopen dat de onzin ophoudt. Please keep giving updates about the retraction itself ! Thank you so much.

Reply

29. **Helga Smilga** says:
November 30, 2020 at 12:15 pm

Thanks to the Bravehearts within the world of science (honest and courageous) the hinges of this utter madness will slowly but surely begin to creak.

Reply

30. **Andre N.** says:
November 30, 2020 at 12:20 pm

Thank you for Work, this is a hope, for all People in the World.
For democracy, the rule of law and freedom.
Especially for the scientists who have made it their mission to work scientifically.
The truth always wins!

Reply

31. **Jos K.** says:
November 30, 2020 at 12:36 pm

Excellent work!

Reply

32. **Dorothee O'Sullivan Burchard** says:
November 30, 2020 at 12:57 pm

Thank you for this excellent work! It will help to rectify the erroneous claims and measures put in place that curtail the human rights of millions of people! Concerns remain as to whether politicians of governments across the globe take this on board. If not, people need to rise up and continue the peaceful fight for their liberties.

Reply

33. **Michiel de Jong** says:
November 30, 2020 at 1:55 pm

Thank you for what you have done for society! We are in debt to you all.

Reply

34. **Gerlinde Hörr** says:
November 30, 2020 at 2:08 pm

Herzlichen Dank für Ihre Mühen! Ein Lichtblick und Hoffnungsschimmer nach acht düsteren Monaten voller Irrsinn! Danke, danke – vielmals!

Reply

35. **Jack AVALONE** says:
November 30, 2020 at 4:17 pm

You all need to spread the #TRUTH on twitter.

#President #TRUMP will surely see it.

Reply

36. **Tanya Sutterfield** says:
November 30, 2020 at 4:33 pm

I am deeply grateful for the service, we are indebted to all involved. I pray this is received and used to change the devastating course we are currently on and cease the criminal actions being perpetrated on humanity.

Reply

37. **Already Provided** says:
November 30, 2020 at 4:45 pm

Great work. A small point:
I'm not quite sure about the first part of your definition of a false positive.

"The definition of false positives is a negative sample, which initially scores positive, but which is negative after retesting with the same test".

–followed by:

"False positives are erroneous positive test-results, i.e. negative samples that test positive."

I would say the second sentence is correct, but not the first sentence. "initially positive but then testing negative negative after retesting" is in my view a case of imprecision (random error) of the test near the limit of detection, not a false positive.

Check out Hedderich, M Sachs L, "Angewandte Statistik" 17th edition, section 4.5 p 186 "Der diagnostische Test", Table 4.6. According to that source, a false positive occurs when:

A sample from a patient *who does not have the disease* gives a positive result in the test.[conditional probability would be P(T+|K-)]

Of course the critical point is defining what the "disease" is. If it is defined as a infectious state attributable to Sars-Cov-2 then you could argue that *all* results from this test are false positive.

Reply

Bobby says:
November 30, 2020 at 5:18 pm

Not cencored, I have answered you and it is visible. but I'm approving it again. We have talked about your semantics concern and we will implement it with your resource links / references in the revised version. The outcome is nevertheless the same.

Reply

Bobby says:
November 30, 2020 at 5:22 pm

We are aware of this semantic problem and it will be implemented in the upcoming revisions. The outcome is nevertheless the same.

Reply

38. **Tatjana Z.** says:
November 30, 2020 at 4:51 pm

Hallo,

danke für eure Arbeit. Ich freue mich immer, wenn Menschen sich auch kritisch mit dem aktuellen Corona-Thema befassen!

Allerdings ist mir eines nicht klar (bitte entschuldigt, wenn ich hier

komplett falsch liege, weil ich fachfremd bin, aber ich musste es zumindest mal adressieren):

Der Hauptkritikpunkt ist doch nicht neu und wurde schon von Dr. Drosten in seinem Podcast vom 18.3. aufgegriffen(09:26 min): https://www.ndr.de/nachrichten/info/16-Coronavirus-Update-Wir-brauchen-Abkuerzungen-bei-der-Impfstoffzulassung,podcastcoronavirus140.html

Natürlich schadet es nicht auf Kritikpunkte mehrfach hinzuweisen, aber bis jetzt hat dieser Hauptkritikpunkt zumindest nicht dazu geführt, dass die Veröffentlichung zurück gezogen wurde.

Schöne Grüße
Tatjana

Reply

Bobby says:
November 30, 2020 at 5:21 pm

Der Hauptkritikpunkt in unserem Review Report sind die nicht zulässigen und “anti-good-laboratory practise” RT-qPCR-Protokoll / Primer Design Unzulänglichkeiten, auf diese geht Drosten in seinem Podcast natürlcih nicht ein. Überhaupt ist Selbstreferenzierung oft ein schlechtes wissenschaftliches Gegen-Argumet.

Reply

39. **Dave Spars** says:
November 30, 2020 at 5:03 pm

Thank you, giving me hope.

Reply

40. **Stephen** says:
November 30, 2020 at 5:12 pm

I am horrified to read this appalling misunderstanding of molecular biology and how PCRs work. This is as bad as HIV denialism. If you don't understand how primer concentrations work and how realtime PCR works and how much of the genome was amplified, please stay at home and let the rest of us get on with dealing with COVID.

Reply

1. **Martin** says:
November 30, 2020 at 7:15 pm

Could you please clarify in detail what has been done wrong by the authors of the review?
Which points in the process of rtPCR did they not understand fully?
Thanks for your answer!

Reply

2. **Peter Looman** says:
November 30, 2020 at 10:44 pm

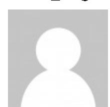
Dit is toch geen serieuze reactie. Ik weet zeker dat de schrijvers open staan als er inhoudelijk fouten aangetoond worden door andere deskundigen. De reactie van Stephen heeft op deze manier de waarde van een gemiddeld Twitterbericht (en die is in mijn mening zeer laag).

Reply

41. **Jabra** says:
November 30, 2020 at 5:12 pm

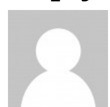
Thanks for your hard work! Hopefully it will be retracted.

Reply

42.  **Dr. Jörg Haberstock** says:
November 30, 2020 at 6:50 pm

Tolle Arbeit, Danke! Wie ist es zu erklären, dass die ganze Welt nirgendwo aktualisierte PCR-Standards mit SOP zu Covid entwickelt hat, wieso greifen die Ringversuche zum Qualitätsmanagement nicht ? Wie kann das alles weltweit und über mehr als 9 Monate unbemerkt geblieben sein? Das macht mich ratlos. Danke für Euren Mut

Reply

43.  **Caro oh oh** says:
November 30, 2020 at 6:51 pm

So, yes, this qPCR is not the best designed one, but due to the circumstances (designed in January as a broad Sars-corovnavirus detection test), this can be understood. Yet, this does not mean that the test does not work. It has been validated a lot by a lot of labs and instances. Every lab has to do a validation/verification of the used tests. Furthermore internal and external controls are taken into account. You can find more details in the paper (and other papers) and also in the news article: https://www.rd.nl/meer-rd/gezondheid/pcr-test-overleeft-stortvloed-aan-kritiek-1.1718351. Next to this, a whole plethora of real-time PCR tests are available on the market.

And yes, having viral RNA in your nose is not the same as being infectious. However, it shows that you have been in contact with the virus. Together with symptoms, this diagnostic test can confirm a diagnosis. Concerning asymptomatic cases, it might be of interest to consider the viral load. It is a valuable point that persons with a low detected viral RNA concentration might not be infectious (yet), or not very well swabbed if no human control

gene is taken along.

I will summarize the other issues I have with this report, as discussing all of them would lead us too far.

1. High primer concentrations: Primer concentrations between100-900 nM are standard (depending on the assay and also the supermix). As the primers contain wobble bases, rather high concentrations make sense. Furthermore, dNTP concentrations are not off from standard conditions.
2. In general wobble bases rather have a negative effect on PCR efficiency (as the correct primer might be exhausted). Here, this seems not the case. On the other hand, it seems that the RdRp assay has a lower sensitivity ((Vogels et al. https://www.nature.com/articles/s41564-020-0761-6), possibly due to a mismatch with some Sars-Cov-2 genomes.
3. Good real-time PCR designs are set-up to detect short fragments (preferably under 150 bp) to obtain a good reaction efficiency. Furthermore, lots of viruses have been sequenced by now (take a look in the NCBI,GISAID,Nextstrain databases for example). The RdRp assay will be transcribed less then the ORF1a transcript, as a frameshift is necessary to transcribe RdRp. Hence, this could theoretically lead to a lower sensitivity of the RdRp assay.
4. The RdRp-assay indeed has not the best design. However, this is a confirmatory assay and it has rather a lower sensitivity (see Voghels et al.). Yes, the E-gene assay might also detect Sars-CoV-1, but this virus is not really going around (and is also causing severe disease).
Off note, concerning melting temperatures, the theoretical Tm calculations should take into account the reaction conditions. Furthermore it is not required to mention Tms, nor GC contents in publications (as you can just copy the primer sequence into an oligo analyzer tool). It is way more useful to validate the annealing temperature in practice (with a gradient PCR for example).
Concerning the primer dimers: as a probe will only detect specific amplicons and not primer dimers, these probably have rather a negative effect on detection.

5. A Cycle Quantification (Cq) threshold is not a unit and is workflow specific. Every lab will have to do its own validation. A Cq value will be dependent on the swab, transport, RNA-extraction, reverse transcription, PCR assay (design, supermix, sample, instrument, plastics) and analysis. You can maybe correlate viral load and time since symptom onset with infectiousness, but not nationwide Cq values (as this will at least be lab dependent, this is not even taking intralab variation into account).
6. I cannot judge about the validation protocol, as probably not every step is described. Melting curves during optimization or sequencing of amplicons is indeed good practice. But again, this assay is a confirmatory assay and has been wet-lab validated. (Gels are IMO for scientists stuck in the nineties and are risks for amplicon contamination.)
7. The test has been validated on negative, positive and other viral controls (read the paper!).
8. The paper and protocol on the WHO website contain a quite well described protocol. Furthermore, each laboratory has to do a proper validation. Btw, it would be unsuitable (in terms of supply chain for example) that every lab is using exactly the same protocol. Reference standards would be useful (but I guess they will come).
9. Funny, as this report is also not peer reviewed, nor has a DOI, and hence, cannot be pubpeer reviewed.
Regardless of this paper, a diagnostic procedure in a lab does not require publication and peer review. It requires wet lab validation.

In conclusion, the design of the RdRp assay is for sure not the best (but there is a good explanation for this). This remains a confirmatory assay after screening with the E-gene assay (or in a multiplex nowadays). The Charite protocol has been extensively validated and remains a valid diagnostic tool.

Reply

Bobby says:
November 30, 2020 at 8:03 pm

Copypaste answers by Prof. Dr. Ulrike Kämmerer:

Quote: So, yes, this qPCR is not the best designed one, but due to the circumstances (designed in January as a broad Sars-corovnavirus detection test), this can be understood. Yet, this does not mean that the test does not work. It has been validated a lot by a lot of labs and instances.

Answer: No, not at the time of publication and supporting the WHO with the Workflow – especially with the knowledge that the Chinese had the PCR and the virus and all informations so far (see literature reference 6 in the report)

Quote: Every lab has to do a validation/verification of the used tests. Furthermore internal and external controls are taken into account.

Answer: No, the real positive control (RNA isolated from the new virus) was not used.

Quote: You can find more details in the paper (and other papers) and also in the news article: https://www.rd.nl/meer-rd/gezondheid/pcr-test-overleeft-stortvloed-aan-kritiek-1.1718351.
Next to this, a whole plethora of real-time PCR tests are available on the market.

Answer: That's not the subject of the criticism of the specific publication.

Quote: And yes, having viral RNA in your nose is not the same as being infectious. However, it shows that you have been in contact with the virus. Together with symptoms (!!! Yes, but nobody tests symptomatic persons only), this diagnostic test can confirm a diagnosis. Concerning asymptomatic cases, it might be of interest to consider the viral load. It is a valuable point that persons with a low detected viral RNA concentration might not be infectious (yet), or not very well swabbed if no human control gene is taken along.

Answer: Yes, but nobody tests symptomatic persons only!

Quote: 1. High primer concentrations: Primer concentrations between100-900 nM are standard (depending on the assay and also the supermix). As the primers contain wobble bases, rather high concentrations make sense.

Answer: Maybe – but not in the case of the E- and N-Gene PCR without wobble bases.

Quote: 2. In general wobble bases rather have a negative effect on PCR efficiency (as the correct primer might be exhausted). Here, this seems not the case. On the other hand, it seems that the RdRp assay has a lower sensitivity ((Vogels et al. https://www.nature.com/articles/s41564-020-0761-6), possibly due to a mismatch with some Sars-Cov-2 genomes.

Answer: Yes, but why the mismatches – the genomes were available at the time of submitting the manuscript and the Vogels paper is from Jul 10 2020.

Quote: 3. Good real-time PCR designs are set-up to detect short fragments (preferably under 150 bp) to obtain a good reaction efficiency.

Answer: Correct.

Quote: Furthermore, lots of viruses have been sequenced by now (take a look in the NCBI,GISAID,Nextstrain databases for example). The RdRp assay will be transcribed less then the ORF1a transcript, as a frameshift is necessary to transcribe RdRp. Hence, this could theoretically lead to a lower sensitivity of the RdRp assay.

Answer: not of interest – its about the publication from January.

Quote: 4. The RdRp-assay indeed has not the best design. However, this is a confirmatory assay and it has rather a lower sensitivity (see

Voghels et al.). Yes, the E-gene assay might also detect Sars-CoV-1, but this virus is not really going around (and is also causing severe disease.

Answer: Yes – but for an "novel" virus the detection system must be highly specific.

Quote: Off note, concerning melting temperatures, the theoretical Tm calculations should take into account the reaction conditions. Furthermore it is not required to mention Tms, nor GC contents in publications (as you can just copy the primer sequence into an oligo analyzer tool). It is way more useful to validate the annealing temperature in practice (with a gradient PCR for example).

Answer: 10 degree difference is a no-go and yes, everybody optimizes the PCR primers for GC and melting temperature before ordering them… so a rubbish argument.

Quote: Concerning the primer dimers: as a probe will only detect specific amplicons and not primer dimers, these probably have rather a negative effect on detection.

Answer: This is correct.

Quote: 5. A Cycle Quantification (Cq) threshold is not a unit and is workflow specific. Every lab will have to do it's own validation. A Cq value will be dependent on the swab, transport, RNA-extraction, reverse transcription, PCR assay (design, supermix, sample, instrument, plastics) and analysis. You can maybe correlate viral load and time since symptom onset with infectiousness, but not nationwide Cq values (as this will at least be lab dependent, this is not even taking intralab variation into account).

Answer: Well – they have "validated" their PCR so they should have shown their PCR data and CT values – and indeed every lab had to adapt the Test inhouse – but this point is missing in the publication – so not ok.

Quote: 6. I cannot judge about the validation protocol, as probably not every step is described. Melting curves during optimization or sequencing of amplicons is indeed good practice. But again, this assay is a confirmatory assay and has been wet-lab validated. (Gels are IMO for scientists stuck in the nineties and are risks for amplicon contamination.)

Answer: no – not wet-lab validated: no clear results for negative and positive controls are shown (including CT).

Reply

1. **Randomer** says:
November 30, 2020 at 10:23 pm

Thank you for confirming the paper by stating three times that the design might not be the best one. Cheers.

Reply

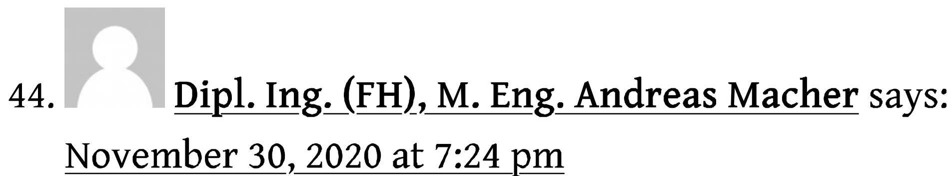
2. **theasdgamer** says:
December 1, 2020 at 2:44 am

"Together with symptoms, this diagnostic test can confirm a diagnosis."

Confirm a diagnosis for what purpose? Adding delays for testing decreases prognosis. Patients are often dilatory about testing and most patients max their viral load on day 3 post symptom onset and maybe contact their primary care physician on day 2 post symptom onset best case. If the doctor won't treat with an antiviral, who cares about any PCR test? A doctor will treat the symptoms of a URTI. If you do treat with an antiviral, you can't wait for the return of test results to begin treating. If the antiviral works, what purpose does the PCR test serve?

And I'm not a physician.

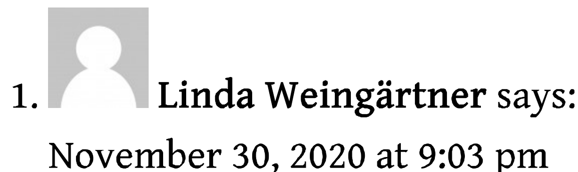
Reply

44.  **Dipl. Ing. (FH), M. Eng. Andreas Macher** says:
November 30, 2020 at 7:24 pm

Um dem nächsten Wahnsinn einer Pseudo-Epedemie vorzubeugen müssen unbedingt rechtzeitig vor den unsicheren Test wirklich sichere Tests für die wahrscheinlich virulenten Virusarten entwickelt werden. Damit nimmt man der Impfindustrie den Spielraum, den sie mit den falsch positiven Tests in dieser Epedemie hatten, für die Zukunft. Als nächstes Target zeichnet sich MERS ab.

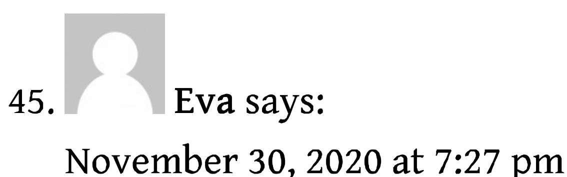
Wenn dieses kompetente Team dafür sorgen würde, dass die relevanten Varianten von MERS wirklich sicher detektiert werden können, wird es nicht noch so eine Panikreaktion in der Bevölkerung geben, wie wir es mit SARS-CoV-2 erleben mussten.

Reply

1.  **Linda Weingärtner** says:
November 30, 2020 at 9:03 pm

Die Panikreaktion der Bevölkerung kam durch die Angstmache der Politik und der Medien zustande.
Die kritischen Stimmen der Experten werden ja bis dato immer noch nicht gehört.

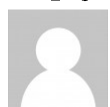
Reply

45.  **Eva** says:
November 30, 2020 at 7:27 pm

Thank you so much for standing up and speaking out. Scientists like you

could restore my faith in science.

Reply

46.  **Ordinary Doc** says:
November 30, 2020 at 8:20 pm

I do not understand the technicalities of pcr testing. I am however an experienced clinician and I understand what I see in my everyday practice. What you are saying seems absolutely correct. False positives++. Well done and good luck.

Reply

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