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Detection of 2019 novel coronavirus (2019-nCoV) by real-time RT-PCR

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Full-Text

Introduction

According to the World Health Organization (WHO), the WHO China Country Office was informed of cases of pneumonia of unknown aetiology in Wuhan City, Hubei Province, on 31 December 2019 [1]. A novel coronavirus currently termed 2019-nCoV was officially announced as the causative agent by Chinese authorities on 7 January. A viral genome sequence was released for immediate public health support via the community online resource virological.org on 10 January (Wuhan-Hu-1, GenBank accession number MN908947 [2]), followed by four other genomes deposited on 12 January in the viral sequence database curated by the Global Initiative on Sharing All Influenza Data (GISAID). The genome sequences suggest presence of a virus closely related to the members of a viral species termed severe acute respiratory syndrome (SARS)-related CoV, a species defined by the agent of the 2002/03 outbreak of SARS in humans [3,4]. The species also comprises a large number of viruses mostly detected in rhinolophid bats in Asia and Europe.

As at 20 January 2020*, 282 laboratory-confirmed human cases have been notified to WHO [5]. Confirmed cases in travellers from Wuhan were announced on 13 and 17 January in Thailand as well as on 15 January in Japan and 19 January in Korea. The extent of human-to-human transmission of 2019-nCoV is unclear at the time of writing of this report but there is evidence of some human-to-human transmission.

Among the foremost priorities to facilitate public health interventions is reliable laboratory diagnosis. In acute respiratory infection, RT-PCR is routinely used to detect causative viruses from respiratory secretions. We have previously demonstrated the feasibility of introducing robust detection technology based on real-time RT-PCR in public health laboratories during international health emergencies by coordination between public and academic laboratories [6-12]. In all of these situations, virus isolates were available as the primary substrate for establishing and controlling assays and assay performance.

In the present case of 2019-nCoV, virus isolates or samples from infected patients have so far not become available to the international public health community. We report here on 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.

Methods

Clinical samples and coronavirus cell culture supernatants for initial assay evaluation

Cell culture supernatants containing typed coronaviruses and other respiratory viruses were provided by Charité and University of Hong Kong research laboratories. Respiratory samples were obtained during 2019 from patients hospitalised at Charité medical centre and tested by the NxTAG respiratory pathogen panel (Luminex, S´Hertogenbosch, The Netherlands) or in cases of MERS-CoV by the MERS-CoV upE assay as published before [10]. Additional samples were selected from biobanks at the Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, at Erasmus University Medical Center, Rotterdam, at Public Health England (PHE), London, and at the University of Hong Kong. Samples from all collections comprised sputum as well as nose and throat swabs with or without viral transport medium.

Faecal samples containing bat-derived SARS-related CoV samples (identified by GenBank accession numbers) were tested: KC633203, Betacoronavirus BtCoV/Rhi_eur/BB98–98/BGR/2008; KC633204, Betacoronavirus BtCoV/Rhi_eur/BB98–92/BGR/2008; KC633201, Betacoronavirus

BtCoV/Rhi_bla/BB98–22/BGR/2008; GU190221 Betacoronavirus Bat coronavirus BR98–19/BGR/2008; GU190222 Betacoronavirus Bat coronavirus BM98–01/BGR/2008; GU190223, Betacoronavirus Bat coronavirus BM98–13/BGR/2008.

All synthetic RNA used in this study was photometrically quantified.

RNA extraction

RNA was extracted from clinical samples with the MagNA Pure 96 system (Roche, Penzberg, Germany) and from cell culture supernatants with the viral RNA mini kit (QIAGEN, Hilden, Germany).

Real-time reverse-transcription PCR

A 25 μL reaction contained 5 μL of RNA, 12.5 μL of 2 × reaction buffer provided with the Superscript III one step RT-PCR system with Platinum Taq Polymerase (Invitrogen, Darmstadt, Germany; containing 0.4 mM of each deoxyribont triphosphates (dNTP) and 3.2 mM magnesium sulphate), 1 μL of reverse transcriptase/Taq mixture from the kit, 0.4 μL of a 50 mM magnesium sulphate solution (Invitrogen), and 1 μg of nonacetylated bovine serum albumin (Roche). Primer and probe sequences, as well as optimised concentrations are shown in Table 1. All oligonucleotides were synthesised and provided by Tib-Molbiol (Berlin, Germany). Thermal cycling was performed at 55 °C for 10 min for reverse transcription, followed by 95 °C for 3 min and then 45 cycles of 95 °C for 15 s, 58 °C for 30 s. Participating laboratories used either Roche Light Cycler 480II or Applied Biosystems ViiA7 instruments (Applied Biosystems, Hong Kong, China).

Table 1. Primers and probes, real-time RT-PCR for 2019 novel coronavirus

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

[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.

Assay/use	Oligonucleotide	Sequence[a]	Concentration[b]
	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.

Protocol options and application notes

Laboratories participating in the evaluation used the TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) with the same oligonucleotide concentrations and cycling conditions. The QIAGEN One-Step RT-PCR Kit was also tested and found to be compatible.

The intended cross-reactivity of all assays with viral RNA of SARS-CoV allows us to use the assays without having to rely on external sources of specific 2019-nCoV RNA.

For a routine workflow, we recommend the E gene assay as the first-line screening tool, followed by confirmatory testing with the RdRp gene assay. Application of the RdRp gene assay with dual colour technology can discriminate 2019-nCoV (both probes positive) from SARS-CoV RNA if the latter is used as positive control. Alternatively, laboratories may choose to run the RdRp assay with only the 2019-nCoV-specific probe.

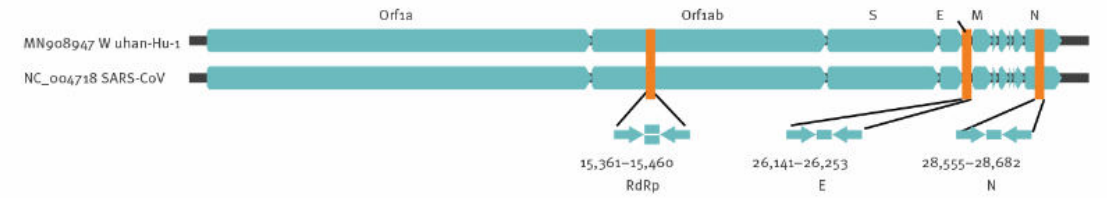
Ethical statement

The internal use of samples for diagnostic workflow optimisation was agreed under the medical ethical rules of each of the participating partners.

Results

Before public release of virus sequences from cases of 2019-nCoV, we relied on social media reports announcing detection of a SARS-like virus. We thus assumed that a SARS-related CoV is involved in the outbreak. We downloaded all complete and partial (if > 400 nt) SARS-related virus sequences available in GenBank by 1 January 2020. The list (n = 729 entries) was manually checked and artificial sequences (laboratory-derived, synthetic, etc), as well as sequence duplicates were removed, resulting in a final list of 375 sequences. These sequences were aligned and the alignment was used for assay design (Supplementary Figure S1). Upon release of the first 2019-nCoV sequence at virological.org, three assays were selected based on how well they matched to the 2019-nCoV genome (Figure 1). The alignment was complemented by additional sequences released independently on GISAID (https://www.gisaid.org), confirming the good matching of selected primers to all sequences. Alignments of primer binding domains with 2019-nCoV, SARS-CoV as well as selected bat-associated SARS-related CoV are shown in Figure 2.

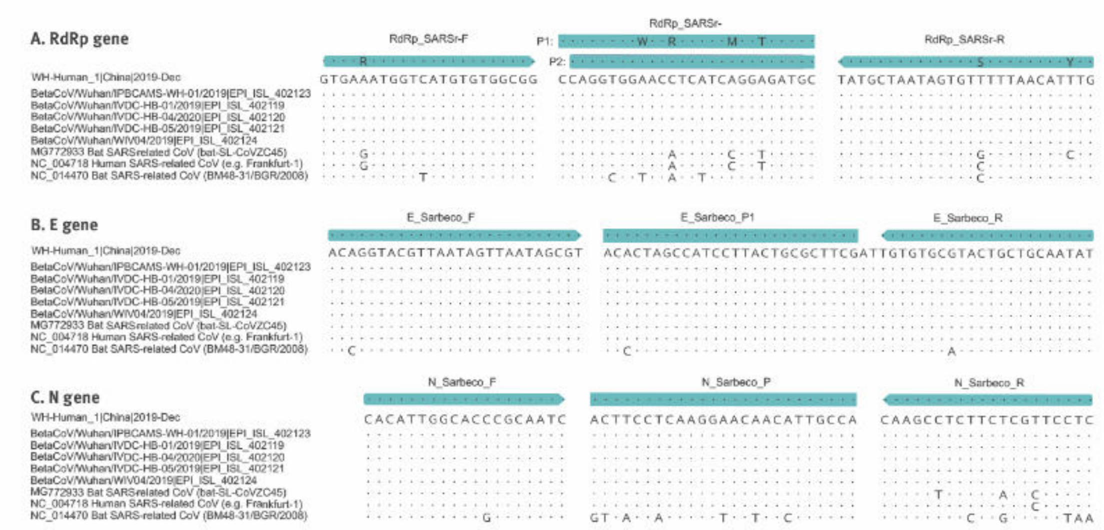
Figure 1. Relative positions of amplicon targets on the SARS coronavirus and the 2019 novel coronavirus genome



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.

Figure 2. Partial alignments of oligonucleotide binding regions, SARS-related coronaviruses (n = 9)

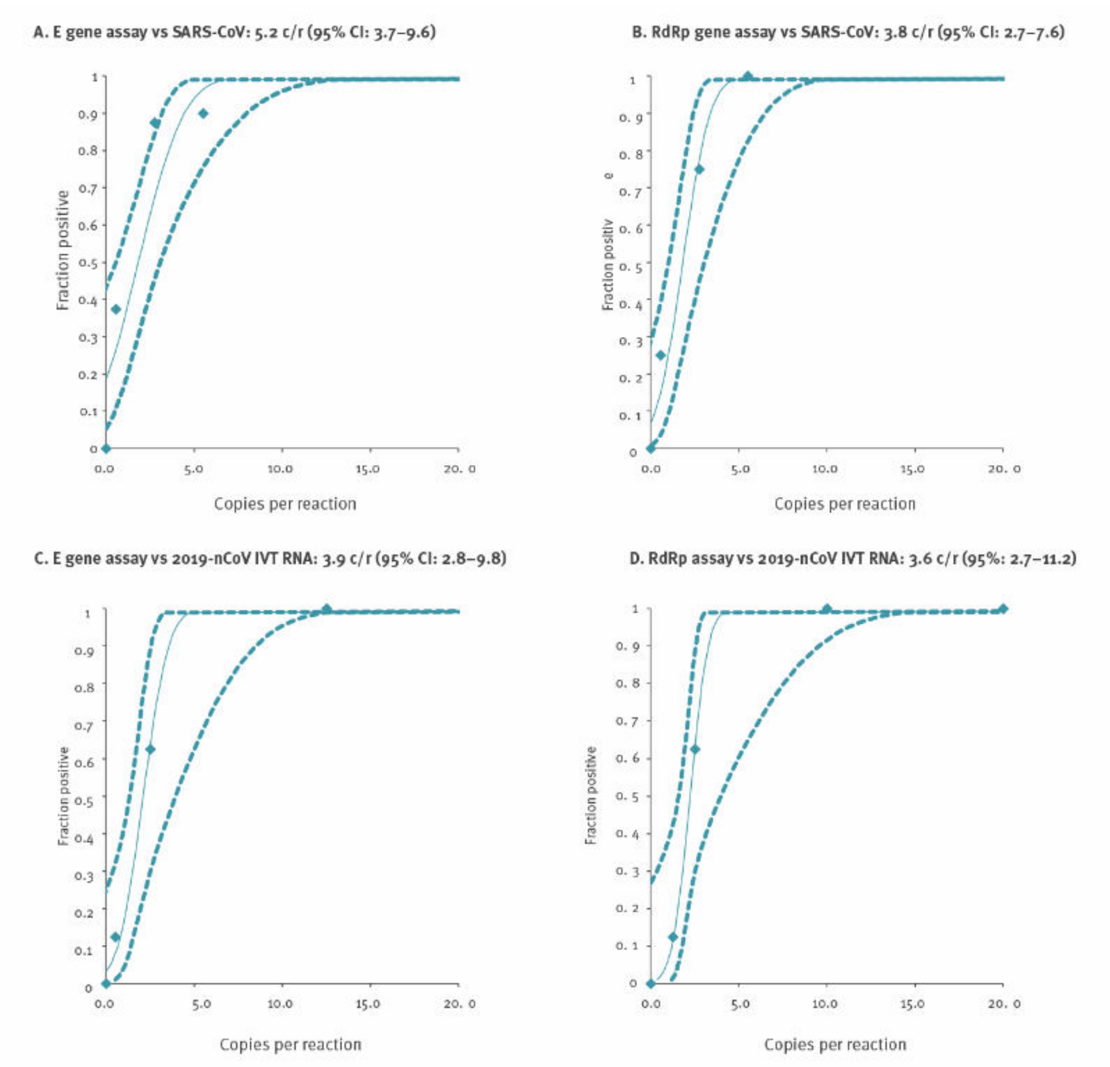


The panels show six available sequences of 2019-nCoV, aligned to the corresponding partial sequences of SARS-CoV strain Frankfurt 1, which can be used as a positive control for all three RT-PCR assays. The alignment also contains a closely related bat virus (Bat SARS-related CoV isolate bat-SL-CoVZC45, GenBank accession number MG772933) as well as the most distant member within the SARS-related bat CoV clade, detected in Bulgaria (GenBank accession number NC_014470). Dots represent identical nucleotides compared with the WH_Human_1 sequence. Nucleotide substitutions are specified. Blue arrows: oligonucleotides as specified in Table 1. More comprehensive alignments can be found in the Supplement.

Assay sensitivity based on SARS coronavirus virions

To obtain a preliminary assessment of analytical sensitivity, we used purified cell culture supernatant containing SARS-CoV strain Frankfurt-1 virions grown on Vero cells. The supernatant was ultrafiltered and thereby concentrated from a ca 20-fold volume of cell culture supernatant. The concentration step simultaneously reduces the relative concentration of background nucleic acids such as not virion-packaged viral RNA. The virion preparation was quantified by real-time RT-PCR using a specific in vitro-transcribed RNA quantification standard as described in Drosten et al. [8]. All assays were subjected to replicate testing in order to determine stochastic detection frequencies at each assay's sensitivity end point (Figure 3A and B). All assays were highly sensitive, with best results obtained for the E gene and RdRp gene assays (5.2 and 3.8 copies per reaction at 95% detection probability, respectively). These two assays were chosen for further evaluation. One of the laboratories participating in the external evaluation used other basic RT-PCR reagents (TaqMan Fast Virus 1-Step Master Mix) and repeated the sensitivity study, with equivalent results (E gene: 3.2 RNA copies/reaction (95% CI: 2.2–6.8); RdRP: 3.7 RNA copies/reaction (95% CI: 2.8–8.0). Of note, the N gene assay also performed well but was not subjected to intensive further validation because it was slightly less sensitive (Supplementary Figure S2)

Figure 3. Determination of limits of detection based on SARS coronavirus genomic RNA and 2019 novel coronavirus-specific in vitro transcribed RNA



CI: confidence intervals; c/r: copies per reaction; IVT: in vitro-transcribed RNA.

A: E gene assay, evaluated with SARS-CoV genomic RNA. B: RdRp gene assay evaluated with SARS-CoV genomic RNA. C: E-gene assay, evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard. D: RdRp gene assay evaluated with 2019-nCoV-specific in vitro-transcribed RNA standard.

The x-axis shows input RNA copies per reaction. The y-axis shows positive results in all parallel reactions performed, squares are experimental data points resulting from replicate testing of given concentrations (x-axis) in parallels assays (eight replicate reactions per point).

Technical limits of detection are given in the panels headings. The inner line is a probit curve (dose-response rule). The outer dotted lines are 95% CI.

Sensitivity based on in vitro-transcribed RNA identical to 2019 novel coronavirus target sequences

Although both assays detected 2019-nCoV without polymorphisms at oligonucleotide binding sites (Figure 2), we additionally generated in vitro-transcribed RNA standards that exactly matched the sequence of 2019-nCoV for absolute quantification and studying the limit of detection (LOD). Replicate reactions were done at concentrations around the detection end point determined in preliminary dilution experiments. The resulting LOD from replicate tests was 3.9 copies per reaction for the E gene assay and 3.6 copies per reaction for the RdRp assay (Figure 3C and D). These figures were close to the 95% hit rate of 2.9 copies per reaction, according to the Poisson distribution, expected when one RNA molecule is detected.

Discrimination of 2019 novel coronavirus from SARS coronavirus by RdRp assay

Following the rationale that SARS-CoV RNA can be used as a positive control for the entire laboratory procedure, thus obviating the need to handle 2019-nCoV RNA, we formulated the RdRp assay so that it contains two probes: a broad-range probe reacting with SARS-CoV and 2019-nCoV and an additional probe that reacts only with 2019-nCoV. By limiting dilution experiments, we confirmed that both probes, whether used individually or in combination, provided the same LOD for each target virus. The specific probe RdRP_SARSr-P2 detected only the 2019-nCoV RNA transcript but not the SARS-CoV RNA.

Detection range for SARS-related coronaviruses from bats

At present, the potential exposure to a common environmental source in early reported cases implicates the possibility of independent zoonotic infections with increased sequence variability [5]. 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. [13] und Muth et al. [14]. 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.

Specificity testing

Chemical stability

To exclude non-specific reactivity of oligonucleotides among each other, causing artificial fluorescent signals, all assays were tested 120 times in parallel with water and no other nucleic acid except the provided oligonucleotides. In none of these reactions was any positive signal detected.

Cross-reactivity with other coronaviruses

Cell culture supernatants containing all endemic human coronaviruses (HCoV)-229E, -NL63, -OC43 and -HKU1 as well as MERS-CoV were tested in duplicate in all three assays (Table 2). For the non-cultivable HCoV-HKU1, supernatant from human airway culture was used. Viral RNA concentration in all samples was determined by specific real-time RT-PCRs and in vitro-transcribed RNA standards designed for absolute quantification of viral load. Additional undiluted (but not quantified) cell culture supernatants were tested as summarised in Table 2. These were additionally mixed into negative human sputum samples. None of the tested viruses or virus preparations showed reactivity with any assay.

Table 2. Tests of known respiratory viruses and bacteria in clinical samples and cell culture preparations for cross-reactivity in 2019 novel coronavirus E and RdRp gene assays (n = 310)

Clinical samples with known viruses	Clinical samples[a]	Virus isolates[b]
HCoV-HKU1	14	1[c]
HCoV-OC43	16	2[d]
HCoV-NL63	14	1[e]
HCoV-229E	18	2[f]
MERS-CoV	5	1[g]
Influenza A(H1N1)pdm09	17	1
Influenza A(H3N2)	16	1
Influenza A (untyped)	11	NA

[a] For samples with multiple viruses detected, the virus with highest concentration is listed, as indicated by real-time PCR Ct value.

[b] Directly quantified or spiked in human negative-testing sputum.

[c] 1×10^5 RNA copies/mL, determined by specific real-time RT-PCR. Isolated from human airway epithelial culture.

[d] 1×10^{10} RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified but spiked in human negative-testing sputum.

[e] 4×10^9 RNA copies/mL, determined by specific real-time RT-PCR.

[f] 3×10^9 RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified spiked in human negative-testing sputum.

[g] 1×10^8 RNA copies/mL, determined by specific real-time RT-PCR.

Clinical samples with known viruses	Clinical samples[a]	Virus isolates[b]
Influenza A(H5N1)	1	1
Influenza A(H7N9)	0	1
Influenza B (Victoria or Yamagata)	31	1
Rhinovirus/enterovirus	31	NA
Respiratory syncytial virus (A/B)	33	NA
Parainfluenza 1 virus	12	NA
Parainfluenza 2 virus	11	NA
Parainfluenza 3 virus	14	NA
Parainfluenza 4 virus	11	NA
Human metapneumovirus	16	NA
Adenovirus	13	1
Human bocavirus	6	NA
Legionella spp.	3	NA
Mycoplasma spp.	4	NA
Total clinical samples	**297**	NA

[a] For samples with multiple viruses detected, the virus with highest concentration is listed, as indicated by real-time PCR Ct value.

[b] Directly quantified or spiked in human negative-testing sputum.

[c] 1×10^5 RNA copies/mL, determined by specific real-time RT-PCR. Isolated from human airway epithelial culture.

[d] 1×10^{10} RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified but spiked in human negative-testing sputum.

[e] 4×10^9 RNA copies/mL, determined by specific real-time RT-PCR.

[f] 3×10^9 RNA copies/mL, determined by specific real-time RT-PCR of one isolate. The other isolate was not quantified spiked in human negative-testing sputum.

[g] 1×10^8 RNA copies/mL, determined by specific real-time RT-PCR.

Exclusivity of 2019 novel coronavirus based on clinical samples pre-tested positive for other respiratory viruses

Using the E and RdRp gene assays, we tested a total of 297 clinical samples from patients with respiratory disease from the biobanks of five laboratories that provide diagnostic services (one in Germany, two in the Netherlands, one in Hong Kong, one in the UK). We selected 198 samples from three university medical centres where patients from general and intensive care wards as well as mainly paediatric outpatient departments are seen (Germany, the Netherlands, Hong Kong). The remaining samples were contributed by national public health services performing surveillance studies (RIVM, PHE), with samples mainly submitted by practitioners. The samples contained the broadest range of respiratory agents possible and reflected the general spectrum of virus concentrations encountered in diagnostic laboratories in these countries (Table 2). In total, this testing yielded no false positive outcomes. In four individual test reactions, weak initial reactivity was seen but 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 but most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study.

Discussion

The present report describes the establishment of a diagnostic workflow for detection of an emerging virus in the absence of physical sources of viral genomic nucleic acid. Effective assay design was enabled by the willingness of scientists from China to share genome information before formal publication, as well as the availability of broad sequence knowledge from ca 15 years of investigation of SARS-related viruses in animal reservoirs. The relative ease with which assays could be designed for this virus, in contrast to SARS-CoV in 2003, proves the huge collective value of descriptive studies of disease ecology and viral genome diversity [8,15-17].

Real-time RT-PCR is widely deployed in diagnostic virology. In the case of a public health emergency, proficient diagnostic laboratories can rely on this robust technology to establish new diagnostic tests within their routine services before pre-formulated assays become available. In addition to information on reagents, oligonucleotides and positive controls, laboratories working under quality control programmes need to rely on documentation of technical qualification of the assay formulation as well as data from external clinical evaluation tests. The provision of control RNA templates has been effectively implemented by the EVAg project that provides virus-related reagents from academic research collections [18]. SARS-CoV RNA was retrievable from EVAg before the present outbreak; specific products such as RNA transcripts for the here-described assays were first retrievable from the EVAg online catalogue on 14 January 2020 (https://www.european-virus-archive.com). Technical qualification data based on cell culture materials and synthetic constructs, as well as results from exclusivity testing on 75 clinical samples, were included in the first version of the diagnostic protocol provided to the WHO on 13 January 2020. Based on efficient collaboration in an informal network of laboratories, these data were augmented within 1 week comprise testing results based on a wide range of respiratory pathogens in clinical samples from natural infections. Comparable evaluation studies during regulatory qualification of in vitro diagnostic assays can take months for organisation, legal implementation and logistics and typically come after the peak of an outbreak has waned. The speed and effectiveness of the present deployment and evaluation effort were enabled by national and European research networks established in response to international health crises in recent years, demonstrating the enormous response capacity that can be released through coordinated action of academic and public laboratories [18-22]. This laboratory capacity not only supports immediate public health interventions but enables sites to enrol patients during rapid clinical research responses.

*Authors' correction

The sentence *As at 20 January 2020, 282 laboratory-confirmed human cases have been notified to WHO* was originally published with a wrong date (*As at 20 January 2019…*). This mistake was corrected on 8 April 2020.

On 29 July 2020 the correct affiliation of Marco Kaiser was added and the remaining affiliations were renumbered.

** Addendum

The Conflict of interest section was updated on 29 July 2020.

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Conflict of interest**

Olfert Landt is CEO of Tib-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for Tib-Molbiol.

Authors' contributions

VMC: Planned and conducted experiments, conceptualised the laboratory work

OL: Planned and conducted experiments, conceptualised the laboratory work

MK: Planned and conducted experiments

RM: Planned and conducted experiments, conceptualised the laboratory work

AM: Planned and conducted experiments, conceptualised the laboratory work

DKWC: Planned and conducted experiments

TB: Planned and conducted experiments

SB: Planned and conducted experiments

JS: Planned and conducted experiments

MLS: Planned and conducted experiments

DGJCM: Planned and conducted experiments

BLH: Planned and conducted experiments

BvdV: Planned and conducted experiments

SvdB: Planned and conducted experiments

LW: Planned and conducted experiments

GG: Planned and conducted experiments

JLR: Contributed to overall study conceptualization

JE: Planned and conducted experiments, conceptualised the laboratory work

MZ: Planned laboratory work, contributed to overall study conceptualization

MP: Planned laboratory work, contributed to overall study conceptualization

HG: Contributed to overall study conceptualization

CR: Planned experiments, conceptualised the laboratory work

MPGK: Planned experiments, conceptualised the laboratory work

CD: Planned experiments, conceptualised the laboratory work, conceptualised the overall study, wrote the manuscript draft.

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Appendix

Supplementary Data

Supplementary Material file-size: 94000.

Supplement

Bilaga 2

Folkhälsomyndigheten

Vilken metod har Folkhälsomyndigheten för att påvisa nukleinsyra av SARS-CoV-2?

Lyssna

Folkhälsomyndigheten använder sig av den metod som WHO rekommenderar (Corman et al). Uppdatering av primrar har varit nödvändig för att öka känsligheten. Om önskemål finns så delar Folkhälsomyndigheten sekvenser för de senaste primrarna.

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Bilaga 3

Folkhälsomyndigheten

Användning av PCR för påvisning av pågående covid-19, en teknisk vägledning

Version 1, 2020-09-24

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Artikelnummer: 20136

Om publikationen

Vägledningen har utarbetats för att beskriva användning av realtids RT-PCR i diagnostik av covid-19 och vilka särskilda beaktanden som bör göras avseende testets prestanda beroende på testningens syfte. I dokumentet används genomgående begreppet PCR som kortform för det längre begreppet realtids RT-PCR.

PCR är en träffsäker och tillförlitlig metod, men då den globala efterfrågan på tester är mycket stor och testningen sker i stor skala finns en risk för att felaktiga resultat kan uppstå, varför kvalitetssäkringsarbetet blir särskilt viktigt. Konsekvensen av felaktiga resultat kan bli omfattande för personen som drabbas, hälso- och sjukvården och samhället i övrigt. I vägledningen lyfts av den anledningen exempel på åtgärder som analyserande laboratorier kan vidta för att minska risken för felaktiga resultat.

Dokumentet vänder sig till kliniska mikrobiologiska laboratorier och externa aktörer som använder PCR för att påvisa pågående covid-19.

Folkhälsomyndigheten

Karin Tegmark Wisell
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Innehåll

Ordlista

Analytisk känslighet	I vägledningen avses hur låga virusnivåer testet klara av att påvisa
Analytisk specificitet	I vägledningen avses testets förmåga att specifikt påvisa nukleinsyran hos viruset som orsakar covid-19
Ct-värde	Mått som indirekt avspeglar virusmängden i ett prov. Ct-värdet är det cykeltal där fluorescenssignalen från PCR-produkten överstiger tröskelvärdet för att räknas som positivt
Cut-off	Vald gräns mellan vad som rapporteras som positivt eller negativt
Diagnostisk känslighet	Mått som anger andelen individer med covid-19 som får ett positivt resultat vid testning. Synonymt med diagnostisk sensitivitet
Diagnostisk specificitet	Mått som anger andelen individer utan covid-19 som får ett negativt resultat vid testning
Extraktion	Metod där man renar fram virusets nukleinsyra från ursprungsprov
Immunitet	Genomgången infektion som resulterar i att personen är helt eller delvis skyddad mot sjukdom vid exponering för viruset som orsakar covid-19
Negativt prediktionsvärde	Andelen negativa testresultat som är sant negativa
Nukleinsyra	Arvsmassa, i vägledningen hos SARS-CoV-2 som orsakar covid-19
Positivt prediktionsvärde	Andelen positiva testresultat som är sant positiva
Prevalens	Mått som anger andelen individer i en population som har eller har haft en sjukdom eller ett tillstånd, i detta fall covid-19

Primer	Kort nukleinsyrafragment som är komplementärt till och binder målsekvens så att amplifiering kan ske
Prob	Kort nukleinsyrafragment som är komplementärt till och binder målsekvens i den bildade PCR-produkten så att påvisning sker
RdRp/ORF1, env, N och S	Namn på regioner och gener som är målsekvenser i flertalet av de PCR-tester som används vid covid-19 diagnostik
Realtids RT-PCR	Realtids Reverse Transcriptase Polymerase Chain Reaction. Metod för att amplifiera och påvisa nukleinsyra i ett prov
SARS-CoV-2	Viruset som orsakar covid-19

Sammanfattning

PCR används för att påvisa pågående covid-19 och det finns såväl egenutvecklade tester som kommersiella att tillgå. PCR-testets prestanda, framför allt dess känslighet och specificitet, är avgörande för att ge tillförlitliga resultat. Olika krav kan ställas på testets prestanda beroende på testningens syfte och det angivna användningsområdet. I vissa sammanhang är hög känslighet viktigast, medan det i andra är testets specificitet som är avgörande.

Prestandan påverkas av många olika faktorer och den utvärderas och säkerställs genom validering och kliniska studier. Även för tester med mycket hög prestanda finns risk för felaktiga resultat i signifikant omfattning när testningen sker i stor skala. PCR har en ökad risk för falskt positiva resultat på grund av bland annat ospecifik amplifiering och bildning av dimers vid höga Ct-värden. En särskild utmaning är därför tolkning av provresultat med höga Ct-värden. Dels på grund av risken för falsk positivitet, men även eftersom virusmängden och därmed Ct-värdet varierar under infektionsförloppet, mellan olika provmaterial, mellan olika tester och beroende på hur väl provtagningen lyckats.

Laboratorieresultat behöver vara så exakta som möjligt. Felaktiga resultat kan ge stora konsekvenser för samhället, hälso- och sjukvården och personen som drabbas. Det är viktigt att laboratoriet vidtar åtgärder som minskar risken för felaktiga resultat. God mikrobiologisk praxis, användning av kontroller som säkerställer att alla analyssteg fungerar på det sätt som definierats i valideringen och rutiner för analys och tolkning av resultat nära testets detektionsgräns är exempel på åtgärder som kan vidtas.

Bakgrund

PCR är den metod som under hela pandemin använts för att påvisa pågående covid-19. Orsaken till att man använder PCR är att virusets nukleinsyra i allmänhet kan påvisas tidigt i infektionsförloppet i provmaterial från de övre luftvägarna. Tidig diagnos är en viktig smittskyddsåtgärd för att minska pandemins utbredning.

Det finns såväl egenutvecklade PCR-tester som ett stort antal olika kommersiella att tillgå för att påvisa pågående covid-19. Pandemins omfattning gör att den globala efterfrågan på tester är stor. PCR är i allmänhet en träffsäker och tillförlitlig metod för diagnostik. Validering och klinisk utvärdering innan ett PCR-test tas i bruk är viktigt för att säkerställa att prestandan är god. Vid testning i stor skala, särskilt i områden där prevalensen är låg eller vid testning av asymtomatiska personer utan klinisk frågeställning, finns en ökad risk för felaktiga resultat (falskt positiva). Denna kan hanteras genom lämpligt valda analys- och kvalitetskontroller. Felaktiga resultat kan få stora konsekvenser för personen som drabbas men framför allt för hälso- och sjukvården och samhället i stort.

Den här vägledningen har utarbetats för att belysa några av de faktorer som påverkar ett tests prestanda, beaktanden som bör göras avseende prestanda beroende på testningens syfte samt vilka åtgärder laboratorier kan vidta för att minska risken för felaktiga resultat.

Syfte

Syftet med vägledningen är att övergripande beskriva faktorer som visat sig vara av särskild relevans för SARS-CoV-2 PCR-testers prestanda och vilka beaktanden som bör göras avseende prestandan beroende på vad syftet med testningen är.

Syftet med vägledningen är även att beskriva relevansen av validering och kliniska studier, orsaker till felaktiga resultat och vilka åtgärder laboratoriet kan vidta för att minska risken för dessa.

Avgränsning

Vägledningen har inte som syfte att ge en fullständig beskrivning av validering och dess omfattning för att fastställa testers prestanda. För vägledning och stöd i sådan frågeställning hänvisas till andra dokument. Se exempelvis dokument utgivet av SWEDAC, ISO 20395:2019 och förordning 2017/746. För ett fåtal smittämnen har EU-kommissionen tagit fram gemensamma tekniska specifikationer, där valideringens omfattning bland annat styrs av testets användningsområde. För SARS-CoV-2 finns ännu ingen teknisk specifikation framtagen, men de som finns för andra smittämnen kan anpassas och vid behov användas som stöd vid validering av tester för SARS-CoV-2.

Vägledningen omfattar inte heller pre-analytiska steg som rör själva provtagningen, exempelvis val av provtagningslokal, provtagningsteknik eller vilka provtagningsmateriel som är lämpliga.

Prestanda hos PCR-tester

PCR är i allmänhet en träffsäker och tillförlitlig metod. Tillförlitligheten påverkas i hög grad av designen av testets primer och prober och vilka av virusets målsekvenser som de utformats för att binda till. Flertalet av de PCR-tester som idag finns tillgängliga amplifierar och detekterar målsekvenser i en eller flera av generna env, N, S, RdRp/ORF1 i SARS-CoV-2 virusgenomet.

Genom att välja målsekvenser i konserverade regioner och i kombination med faktorer som god primer- och probdesign, optimerade reagens, optimerad temperaturprofil och utprovat cykelantal är det möjligt att uppnå mycket hög analytisk specificitet och känslighet i ett test. Testets analytiska känslighet och därmed detektionsgräns påverkas förutom av faktorerna som nämns ovan, exempelvis även av kvalitén i extraktion av nukleinsyra, förekomst av inhiberande substanser som saltrester och cellpartiklar i prov eller provtagningsmateriel och av eventuella sekundärstrukturer i primers, prober eller målsekvenser. Pre-analytiska faktorer som provförvaring och provtransport (tid och temperatur) kan också påverka testresultatet. Bristande valideringar och studier under utvecklingen av ett test kan leda till bristande kännedom om och förståelse för testets prestanda, med följden att prestandan blir sämre.

Olika krav kan ställas på testets prestanda beroende på testningens syfte och det angivna användningsområdet. I en situation där det är viktigt att hitta alla som är smittade är det viktigt att testet har hög analytisk känslighet, det vill säga klarar av att hitta låga virusnivåer i det provmaterial som testas. För att ge hög diagnostisk känslighet krävs hög analytisk känslighet, men även att provet är taget i ett läge av infektionsförloppet då virusets nukleinsyra finns i det provmaterial som testas. Hög känslighet är särskilt viktigt för ett test som används för att diagnosticera patienter med misstänkt covid-19 i behov av slutenvård. Vid storskalig testning i en befolkning där prevalensen är låg kan man acceptera ett test med något lägre känslighet medan det är viktigt att testet har mycket hög specificitet för att det positiva prediktionsvärdet ska bli högt, se tabell 1 nedan. Om ett test med lägre specificitet används kommer en större andel av dem som får ett positivt resultat att få ett felaktigt sådant vilket kan få stora konsekvenser för personen som drabbas, för hälso- och sjukvården och för samhället i stort.

Valideringens omfattning styrs bland annat av testets användningsområde. Ett test vars primära syfte är att användas vid screening behöver innan det tas i bruk exempelvis ha utvärderas på ett mycket stort antal negativa prover, se vidare i dokument som länkats till i avsnittet *Avgränsningar*. Det är viktigt att tydliggöra för beställaren för vilka patientkategorier och i vilket syfte testet är ämnat att användas. För kommersiella CE-IVD tester framgår det av bruksanvisningen.

Tabell 1. Exempel på hur resultatet varierar med testets specificitet och prevalensen i befolkningen. 10 000 personer testas och testets analytiska känslighet är 99 procent. Antal personer är avrundade till heltal i tabellen

Prevalens	**0,1 %**			**0,5 %**			**1 %**			**5 %**		
Testets specificitet i procent	100,0	99,9	99,5	100,0	99,9	99,5	100,0	99,9	99,5	100,0	99,9	99,5
Antal positiva utifrån förekomst	10	10	10	50	50	50	100	100	100	500	500	500
Antal negativa utifrån förekomst	9990	9990	9990	9950	9950	9950	9900	9900	9900	9500	9500	9500
Sant positivt resultat i test	10	10	10	50	50	50	99	99	99	495	495	495
Sant negativt resultat i test	9990	9980	9940	9950	9940	9900	9900	9890	9851	9500	9491	9453
Falskt positivt resultat i test	0	10	50	0	10	50	0	10	50	0	10	48
Falskt negativt resultat i test	0	0	0	1	1	1	1	1	1	5	5	5
Positivt prediktionsvärde i procent	100	49,8	16,5	100	83,3	49,9	100	90,9	66,7	100	98,1	91,2

Källor till felaktiga resultat och åtgärder för att begränsa dem

Otillräcklig validering av ett tests prestanda innan det tas i bruk kan leda till ökad risk för felaktiga resultat, både falskt negativa och falskt positiva. Utöver den validering som ingår under utvecklingen av ett test är kliniska studier viktiga. Vid kliniska studier kan man bland annat utvärdera när under infektionsförloppet och för vilka provmaterial testet är användbart, att prestandan är tillräcklig i ett kliniskt sammanhang och att de inställningar som gjorts för att ge ett tillförlitligt resultat är lämpliga. Det kan exempelvis handla om inställningar för Ct cut-off, gränsen mellan vad som räknas som positivt och negativt. Denna påverkar både testets diagnostiska känslighet och diagnostiska specificitet. Ju lägre vald cut-off desto högre diagnostisk specificitet, men sämre diagnostisk känslighet som följd.

Falskt positiva resultat

I en situation där prevalensen av covid-19 är låg och ett stort antal asymtomatiska personer testas finns en risk för falskt positiva resultat även om testets specificitet är mycket hög, se tabell 1.

Kvarstående PCR-positivitet finns rapporterad upp till månader efter genomgången covid-19, vilket kan leda till att ett positivt resultat felaktigt tolkas som en ny infektion medan det i själva verket är en tidigare som mäts. För tidigare PCR-bekräftat fall är förnyad provtagning i allmänhet inte indikerat inom sex månader från föregående testtillfälle, se vidare i Provtagningsindikation för nukleinsyrapåvisning vid covid-19.

Vanliga orsaker till falskt positiva resultat är kontaminering av prov eller reagens med tidigare amplifierad nukleinsyra (amplikon) och korskontamination från närliggande positivt prov eller positiv kontroll. Prover med mycket höga virusnivåer och därmed låga Ct-värden förekommer vid covid-19 diagnostik. Sådana prover innebär en ökad risk för korskontamination vid hantering eller under själva PCR-reaktionen. En annan källa är korskontaminering som kan uppstå mellan prover under uppsamling, transport och provhantering i laboratoriet. Storskalig produktion av syntetiska kontroller kan också leda till kontamination i produktion och produktionsmiljö. Generös användning av negativa kontroller som omfattar alla delsteg av analysflödet och en strukturerad plan för bekräftande testning vid osäkra resultat bidrar till att laboratoriekontaminering upptäcks och att prover inte felaktigt svaras ut som positiva.

PCR har en ökad risk för falskt positiva resultat vid höga Ct-värden. Efter ett högt antal cykler kan slumpmässig, ospecifik amplifiering uppstå utan att primers och prob har perfekt passform till målsekvensen. Det kan också ske på grund av dimer-bildning där det är testets primers och prober som ger upphov till en positiv signal. Ct-intervallet där slumpmässig amplifiering kan uppstå varierar mellan olika tester. Testresultat som väger samman och beaktar utfallet från fler än en målsekvens har i allmänhet en lägre risk för falskt positiva resultat på grund av denna typ av artefakt än tester som använder en målsekvens.

Även instabilitet hos proben kan ge upphov till falskt positiva resultat genom att risken för bakgrundssignal ökar. Felaktigt förvarade reagens, eller låg kvalitet hos proben orsakad av exempelvis orenheter ökar risken för probdegradering.

Laboratorier som utför PCR-analys för covid-19 bör etablera rutiner för hur resultat med höga Ct-värden hanteras, för definition av höga Ct-värden och hantering se avsnitt *Hantering av svagt positiva prover med höga Ct-värden*.

Falskt negativa resultat

Tester med lägre känslighet har en ökad risk för falskt negativa resultat. Risk för falskt negativa resultat finns också om provet tas för tidigt i infektionsförloppet, då personen befinner sig i inkubationsfasen.

En annan orsak till falskt negativa resultat kan vara att infektionsfokus skiftat en tid efter insjuknandet så att virusets nukleinsyra inte längre kan påvisas i det provmaterial som testas. Falskt negativa resultat kan också uppstå på grund av inhibition, felaktig eller dålig provtagning, eller om virusets nukleinsyra brutits ner under transport eller lagring.

Provförväxling under uppsamling, transport och provhantering i laboratoriet är ytterligare en orsak till både falskt positiva och falskt negativa resultat.

Lämpligt valda analyskontroller som identifierar inhibition eller prover med låg kvalitet av annan orsak minskar risken för de flesta falskt negativa resultat.

Ct-värde i relation till infektionsförloppet

Virusmängden och därmed Ct-värdet kan variera under infektionsförloppet. Låga Ct-värden ses oftast i den tidigare delen av förloppet då den provtagnes smittsamhet är som störst medan höga Ct-värden ses i den senare delen och kan kvarstå upp till månader efter genomgången infektion.

Ct-värdet varierar även beroende på vilket provmaterial som testas, hur väl provtagningen lyckats och mellan olika tester. Att sätta en gräns för vad som räknas som ett högt Ct-värde bör avgöras av laboratoriet utifrån det test som används och den eller de provtyper som är aktuella. Ett lämpligt riktmärke är Ct-värden som ligger i närheten av respektive tests detektionsgräns, alternativt det område där resultat från valideringen visar att testets reproducerbarhet är lägre.

Hantering av svagt positiva prover med höga Ct-värden

Alla laboratorier rekommenderas att etablera rutiner för hantering av prover med höga Ct-värden. Rekommenderad hantering i fallande ordning är:

- Ny extraktion av ursprungligt prov och analys med ett test som använder andra målsekvenser eller har en högre känslighet
- Ny extraktion av ursprungligt prov och analys med samma test som vid det föregående analystillfället
- Ny analys från tidigare extraherat material vid misstanke om kontamination i PCR-reaktionen. Antingen med ett test som använder andra målsekvenser eller har en högre känslighet, eller med samma test som vid det föregående analystillfället

I de fall testresultaten från de två analysomgångarna inte är samstämmiga rekommenderas att provet svaras ut som ”Ej bedömbart” med rekommendation om att ett nytt prov tas.

Notera att det för kommersiella CE-IVD tester inte är tillåtet att frångå de inställningar (exempelvis Ct cut-off, fluorescens cut-off) som fastställts av tillverkaren. Den prestanda som uppges gäller under de förhållanden som tillverkaren specificerat. Om ett test modifieras utan tillverkarens medgivande eller används för testning på annan patientkategori än den testet är godkänt för är det närmast att betrakta som egentillverkning. Vårdgivaren övertar då det fulla ansvaret för testet från tillverkaren.

Konsekvenser av felaktiga resultat

Ett falskt negativt resultat medför ökad risk för smittspridning till och mellan personal och andra patienter/omsorgstagare inom hälso- och sjukvård samt omsorg. Även i samhället i övrigt medför ett falskt negativt resultat risk för smittspridning.

Ett falskt positivt svar får för personen som drabbas konsekvenser i form av smittskyddsåtgärder och skyddsföreskrifter som vid ett korrekt resultat inte hade varit nödvändiga, ekonomisk påverkan på grund av onödig sjukfrånvaro samt antagen immunitet med risk för förändrat smittskyddsbeteende och bristande förståelse för relevansen av provtagning vid faktisk covid-19. Inom hälso- och sjukvård kan ett falskt positivt svar medföra att felaktiga beslut tas gällande kohortvård, behov av förnyad provtagning vid symtom på covid-19 och vid smittspårning.

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13. REGULATION (EU) 2017/746 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 5 April 2017 on in vitro diagnostic medical devices and repealing Directive 98/79/EC and Commission Decision 2010/227/EU
14. COMMISSION DECISION of 27 November 2009 amending Decision 2002/364/EC on common technical specifications for in vitro diagnostic medical devices

Folkhälsomyndigheten är en nationell kunskapsmyndighet som arbetar för en bättre folkhälsa. Det gör myndigheten genom att utveckla och stödja samhällets arbete med att främja hälsa, förebygga ohälsa och skydda mot hälsohot. Vår vision är en folkhälsa som stärker samhällets utveckling.

Folkhälsomyndigheten

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www.folkhalsomyndigheten.se

Folkhälsomyndigheten

Kravspecifikation för upphandling av laboratorieanalys för påvisning pågående covid-19 infektion

Giltigt från: 2020-08-13

Syfte

Syftet med denna specifikation är att klargöra vilka krav som finns på laboratoriet vid utförandet av PCR-baserad laboratorieanalys för covid-19. Kraven är de som Folkhälsomyndigheten ställer inför upprättande av avtal. Regioner kan skriva egna avtal med andra laboratorier och ställa andra krav.

Kravspecifikation

Det övergripande kravet är att verksamheten ska vara kvalitetssäker och utföras på ett patientsäkert sätt. Nedan finns listat vilka krav som Folkhälsomyndigheten ställer för att dessa ska uppnås. Ytterligare aspekter kan behöva hanteras för att uppnå detta och listan utesluter inte att verksamhetsutövaren behöver vidta ytterligare åtgärder för att säkerställa patientsäkerheten.

Laboratoriet ska utföra uppdraget inom de författningar som gäller för klinisk laboratorieverksamhet. Stöd finns i vägledning för diagnostik av covid-19.

Aktuella författningar inkluderar: patientsäkerhetslagen, smittskyddslagen, arbetsmiljöverkets föreskrifter, patientdatalagen, EUs dataskyddsförordning och biobankslagen.

Laboratoriet ska ha ett kvalitetsledningssystem för att upprätthålla ett kvalitetssäkert arbetssätt för att främja patientsäkerheten.

Laboratoriets kvalitetsledningssystem ska säkerställa korrekthet i handhavandet av prov och data i alla steg från det att provet eller beställningen inkommer, vilket som infaller först, tills prov är utsvarat och eventuellt anmält till SmiNet.

Rutiner och andra dokument som ingår i kvalitetsledningssystemet ska vara skriftliga, fastställda och det ska tydligt framgå vilken version som är giltig. Laboratoriets kvalitetsledningssystem ska omfatta, men inte begränsas till, rutiner för:

- att ha spårbarhet i alla steg från det att provet eller beställningen inkommer, vilket som infaller först, tills prov är utsvarat och eventuellt anmält till SmiNet. Med spårbarhet menas bland annat att det ska framgå vem som gjort vad och när, samt vilka reagens och vilken utrustning som använts.

Folkhälsomyndigheten 2020. Artikelnummer 20098-1, revidering 1

- att hantera och registrera inkomna remisser
- att märka och hantera prov
- att prov med oklar identitet inte analyseras, om inte identiteten kan säkerställas på ett av medicinskt ansvarig godkänt sätt
- behandling av personuppgifter
- att hantera laboratoriedata, inklusive att säkerställa att data hanteras på ett säkert sätt så att inte obehöriga får tillgång
- att säkerställa informationssäkerhet i de fall IT-system används, bland annat att dataöverföring är korrekt och att obehöriga inte får tillgång
- att utrustning kalibreras och/eller kontrolleras på lämpligt sätt för att säkerställa funktion
- att om analysen ger oklart resultat tydligt markera det i provsvaret
- att leverera provsvar till behandlande läkare
- att skyndsamt anmäla positiva fall till smittskyddsläkaren, och Folkhälsomyndigheten via SmiNet
- att hantera biobank om prover sparas mer än 2 månader efter avslutad analys
- att rapportera, åtgärda och följa upp avvikelser (oönskade händelser)
- att anmäla personuppgiftsincidenter till Datainspektionen
- att anmäla till IVO vid händelse som har eller kunde ha medfört allvarlig
- vårdskada
- tystnadsplikt för legitimerad personal eller tystnadsförbindelse för övrig personal som har tillgång till patientuppgifter
- att säkerställa att medarbetare har rätt kompetens för sina arbetsuppgifter och att dokumentera vilka medarbetare som har behörighet att utföra analys av prover och andra kritiska rutiner
- att medicinsk delegering görs om medarbetare som inte är läkare svarar ut

Laboratoriet ska:

- ha anmält verksamheten till IVO enligt patientsäkerhetslagen
- ha anmält verksamheten till IVOs biobanksregister, om prov sparas mer än två månader efter avslutad analys
- ha en medicinskt ansvarig läkare lokalt på plats. Medicinskt ansvarig läkare ska bland annat handlägga tolkningsproblematik eller medicinska frågor som rör analyserna, samt vid behov delegera behörighet för utsvar.
- ha validerat den eller de laboratoriemetoder som används enligt checklista för stöd vid införande av nukleinsyrapåvisning av SARS-CoV-2.
- delta i externt kvalitetskontrollprogram regelbundet, minst en gång per år, via ett oberoende organ

- erbjuda analyser till regioner där det finns behov av utökad kapacitet
- ha möjlighet att utföra minst 5000 analyser/vecka
- svara ut minst 95 % av proverna inom 24 timmar från ankomst till
- laboratoriet vid jämnt provflöde. Vid ojämnt provflöde kan svarstiderna förlängas, dock aldrig längre än 48 timmar från ankomst
- ha en digital anslutning till exempelvis 1177 Egen provhantering eller annan digital beställningsfunktion som regionen avtalat att använda, eller
- ha en tidsatt och av Folkhälsomyndigheten godkänd plan för detta genomföra riskbedömning enligt AFS 2018:4
- vid behandling av personuppgifter tillämpa dataskyddsförordningen samt lagen med kompletterande bestämmelser till EU:s dataskyddsförordning

Bilaga 5

Läkartidningen DEBATT

Ct-värden bör beaktas vid tolkning av PCR-resultat

Magnus Lindh, sektionschef, överläkare, professor, klinisk mikrobiologi, Sahlgrenska universitetssjukhuset, Göteborg

Nästan all påvisning av sars-cov-2 görs med realtids-PCR. En målgen kopieras då genom upphettning och nedkylning i 45 cykler och varje reaktion följs med en kamera. PCR-cykeln då en positiv reaktionsfluorescerande signal först påvisas kallas Ct-värde (cykeltröskelvärde). Ett lågt Ct-värde betyder att viruskoncentrationen i provet är hög. Grovt räknat motsvarar Ct-värden på 17, 27 respektive 37 förekomst av 1 miljard, 1 miljon respektive 1 000 viruskopior i provet. Att så stora skillnader förekommer i kliniska prov kan sannolikt förklara de stora variationer i smittspridning som förekommer [1-2]. De flesta mikrobiologiska laboratorier i Sverige rapporterar i dagsläget inte Ct-värden till den som beställt analysen.

Vissa smittade blir PCR-negativa inom en vecka efter symtomdebut, hos andra kan sars-cov2-RNA påvisas i nasofarynxprov 2-4 veckor, ibland ännu längre [3-4]. Ett positivt test kan alltså avspegla en infektion som nästan läkt ut och behöver inte innebära smittrisk. Omvänt gäller att ett negativt resultat inte utesluter att patientens symtom beror på covid-19, särskilt inte om provet tagits en vecka eller mer efter symtomdebut.

Att resultatet av ett PCR-prov kan vara negativt hos en individ med covid-19-orsakad andnöd betyder inte att metoden har låg sensitivitet utan avspeglar att denna komplikation i regel uppträder sent i förloppet då virusreplikation kan saknas eller vara mycket låggradig i de övre luftvägarna [3-5]. Vid denna kliniska misstanke bör man ta nytt prov, om möjligt från nedre luftvägarna, och dessutom göra antikroppstest.

Falskt positiva resultat är vanligen ett litet problem vid realtids-PCR, men kan få stor betydelse vid storskalig testning av individer utan misstänkt infektion. Orsaken kan vara kontamination, alltså att virus överförs från prov med mycket hög virusmängd till annat prov (vid manuell provhantering eller i robotar), eller en ospecifik signal som kan genereras om utformningen av söksekvenserna (primrar och probe) inte varit optimal. Falskt positiv reaktion visar sig i regel som ett Ct-värde över 37 och är ofta omöjlig att skilja från sant positiv reaktion i ett prov med låg virusnivå.

Vid covid-19-diagnostik kan falskt positiva resultat få betydande konsekvenser. De kan generera missvisande statistik som påverkar preventiva strategier och leda till anmälningar enligt smittskyddslagen samt resurskrävande smittspårning. På sjukhus kan personal i onödan behöva använda skyddsutrustning och patienter i onödan isoleras och kanske få suboptimal vård. Personer som testats positivt måste följa smittskyddsföreskrifter, vilket kan påverka arbete och semestrar. De som med ledning av ett positivt PCR-test tror sig immuna riskerar att smitta personer i riskgrupper om de vid en senare tidpunkt infekteras. Sådana fall kan då felaktigt tolkas som reinfektion.

På klinisk mikrobiologi, Sahlgrenska, tolkas PCR-resultatet med ledning av Ct-värdet, vilket rapporteras till beställande läkare. Ct-värdet kan användas för att skatta risken för att resultatet representerar en falskt positiv reaktion eller för att bedöma smittrisk [6]. Genom att prov med Ct-värden över 38 besvaras som negativa kan effekter av falskt positiva resultat undvikas. Resultat med Ct 36–38 bedöms som inte säkert positiva och den som provtagits bedöms som inte smittsam. Vid Ct-värden under 20 poängteras den stora smittrisken i det skriftliga svaret, och om det bedöms motiverat av kliniska uppgifter meddelas svaret per telefon så att smittspridning snabbt kan förhindras och exponerade personer testas.

Avsaknaden av standardisering och inverkan av PCR-effektivitet och provets kvalitet brukar anföras mot användning av Ct-värden. Enligt vår bedömning är dock den kliniska nyttan så stor att Ct-värden bör beaktas vid tolkning av resultatet och rapporteras till beställaren. Vår erfarenhet är att de flesta beställare delar denna uppfattning och att de snabbt lär sig Ct-värdets innebörd och användning.

PUBLICERAD:

Läkartidningen 38-39/2020
Lakartidningen.se 2020-09-16

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2 **Kommentera**

Skriv kommentar...

Hög CT = Ej smittsam?

Intressant att läsa hur andra labb/sjukhus hanterar CT-värden, jag tycker dock att det känns riskabel att patienter med svag positiva PCR (dvs Ct 36-38) bedöms som inte smittsamma. Många faktorer kan påverka Ct-värden: – Inhibitorer kan påverka PCR-reaktionens effektivitet, så det krävs en internkontroll som noga måste kontrolleras för varje prov/analys för att säkerställa att ett svagt resultat inte beror på inhibition. Görs det hos er på Sahlgrenska? – En väldig viktig faktor är provtagningen. I början av pandemin testades patienter här i Umeå med två olika prover som togs i nasopharynx och svalg, och vi hade fall där patienter... Läs mer »

Hinnerk Eilers, Molekylärbiolog, Norrlands Universitetssjukhus, Umeå

september 21, 2020 10:06

Intressant!

Tack för intressant inlägg! Jag har sett en hel del publicerat i litteraturen om klinisk relevans av Ct värden för flera olika virus (nu senast massor om SARS-CoV-2) men jag var inte bekant med att man svarade ut det enskilda Ct värdet.

Jag förutsätter att det validerats på olika sätt internt (spädningsserier?) och att interna kontroller är med i varje omgång.

Standardisering mellan labb är ju omöjligt då alla använder olika kit, maskiner, reaktionsvolym. Har ni sett någon variabilitet mellan olika batchar av mastermix, primer, probe t.ex.? Har ni jämfört med andra labb (Equalis)?

Marcus Fredriksson Sundbom, Underläkare/doktorand/biomedicinare (master), AnOpIVA NUS/UmU

september 21, 2020 15:23

ADRESS: Läkartidningen, Box 5603, 114 86 Stockholm TELEFON: 08-790 33 00 E-POST: webmaster@lakartidningen.se
CHEFREDAKTÖR OCH ANSVARIG UTGIVARE: Pär Gunnarsson
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Bilaga 6

Acórdãos TRL

Acórdão do Tribunal da Relação de Lisboa

Processo:	1783/20.7T8PDL.L1-3
Relator:	MARGARIDA RAMOS DE ALMEIDA
Descritores:	HABEAS CORPUS INTERESSE EM AGIR SARS-COV-2 TESTES RT-PCR PRIVAÇÃO DA LIBERDADE DETENÇÃO ILEGAL
Nº do Documento:	RL
Data do Acordão:	2020-11-11
Votação:	UNANIMIDADE
Texto Integral:	S
Texto Parcial:	N
Meio Processual:	RECURSO PENAL
Decisão:	NEGADO PROVIMENTO

Sumário:

I. A ARS não pode recorrer de uma decisão que ordenou a libertação imediata de quatro pessoas, por detenção ilegal, no âmbito de um processo de *habeas corpus* (artº 220 als. c) e d) do C.P.Penal), pedindo que seja validado *o confinamento obrigatório dos requerentes, por serem portadores do vírus SARS-CoV-2 (A….) e por estarem em vigilância activa, por exposição de alto risco, decretada pelas autoridades de saúde (B…, C…. e D…..)* por não ter legitimidade, nem interesse em agir.

II. O pedido formulado seria, igualmente, manifestamente improcedente porque:

A. A prescrição e o diagnóstico são actos médicos, da exclusiva responsabilidade de um médico, inscrito na Ordem dos Médicos (Regulamento n.º 698/2019, de 5.9).

Assim, a prescrição de métodos auxiliares de diagnóstico (como é o caso dos testes de detecção de infecção viral), bem como **o diagnóstico quanto à existência de uma doença, relativamente a toda e qualquer pessoa, é matéria que não pode ser realizada por Lei, Resolução, Decreto, Regulamento ou qualquer outra via normativa**, por se tratarem de actos que o nosso ordenamento jurídico reserva à competência exclusiva de um médico, sendo certo que este, no aconselhamento do seu doente, deverá sempre tentar obter o seu consentimento esclarecido (nº1 do artº 6º da Declaração Universal sobre Bioética e Direitos Humanos).

B. No caso que ora nos ocupa, não há qualquer indicação nem prova, de tal diagnóstico ter sido efectivamente realizado por profissional habilitado nos termos da Lei e que tivesse actuado de acordo com as boas práticas médicas. Efectivamente, o que decorre dos factos dados como assentes, é que nenhum dos requerentes foi sequer visto por um médico, o que se mostra francamente inexplicável, face à invocada gravidade da infecção.

C. O único elemento que consta nos factos provados, a este respeito, é a realização de testes RT-PCR, sendo que um deles apresentou um resultado positivo em relação a uma das requerentes.

D. Face à actual evidência científica, esse teste mostra-se, só por si, incapaz de determinar, sem margem de dúvida razoável, que tal positividade corresponde, de facto, à infecção de uma pessoa pelo vírus SARS-CoV-2, por várias razões, das quais destacamos duas (a que acresce a questão do *gold standard* que, pela sua especificidade, nem sequer abordaremos):
Por essa fiabilidade depender do número de ciclos que compõem o teste;
Por essa fiabilidade depender da quantidade de carga viral presente.
III. **Qualquer diagnóstico ou qualquer acto de vigilância sanitária** (como é o caso da determinação de existência de infecção viral e de alto risco de exposição, que se mostram abrangidas nestes conceitos) **feitos sem observação médica prévia** aos pacientes e sem intervenção de médico inscrito na OM (que procedesse à avaliação dos seus sinais e sintomas, bem como dos exames que entendesse adequados à sua condição), **viola o Regulamento n.º 698/2019, de 5.9, assim como o disposto no artº 97 do Estatuto da Ordem dos Médicos, sendo passível de configurar o crime de usurpação de funções, p. e p. pelo artº 358 al.b), do C.Penal.**
IV. Qualquer pessoa ou entidade que profira uma ordem, cujo conteúdo se reconduza à privação da liberdade física, ambulatória, de outrem (qualquer que seja a nomenclatura que esta ordem assuma: confinamento, isolamento, quarentena, resguardo profiláctico, vigilância sanitária etc), **que se não enquadre nas previsões legais, designadamente no disposto no artº 27 da CRP**, **estará a proceder a uma detenção ilegal**, porque ordenada por entidade incompetente e porque motivada por facto pelo qual a lei a não permite.
(Sumário elaborado pela relatora)

Decisão Texto Parcial:

Decisão Texto Integral:

Acordam em conferência na 3ª secção Criminal do Tribunal da Relação de Lisboa

*

I – relatório

1. Por decisão de 26-08-2020, foi concedido provimento ao pedido de *habeas corpus* formulado, **por se mostrar ilegal a sua detenção**, determinando-se **a restituição imediata à liberdade dos Requerentes SH__SWH___, AH___ e NK___.**

2. Veio então a **AUTORIDADE REGIONAL DE SAÚDE,** representada pela Direcção Regional da Saúde da Região Autónoma dos Açores apresentar recurso de tal decisão, pedindo a final que seja validado *o confinamento obrigatório dos requerentes, por serem portadores do vírus SARS-CoV-2 (AH___) e por estarem em vigilância activa, por exposição de alto risco, decretada pelas autoridades de saúde (SH__, SWH__ e NK___).*

4. O recurso foi admitido.

5. O Mº Pº, na sua resposta, defende que o presente recurso deve ser considerado improcedente.

6. Neste tribunal, o Exº PGA apôs visto.

II – ponto prévio.

Uma vez que o recurso interposto pela recorrente deve ser rejeitado, o tribunal limitar-se-á, nos termos dos n.ºs 1, alínea a), e 2 do artigo 420.º do Código de Processo Penal, a especificar sumariamente os fundamentos da decisão.

III – fundamentação.

1. A decisão proferida pelo tribunal “a quo” tem o seguinte teor:

Factos provados:

1. Em 01/08/2020 os requerentes chegaram à ilha de São Miguel, provindos por avião da República Federal da Alemanha, onde, nas 72 (setenta e duas) horas anteriores à chegada, tinham realizado um teste ao COVID19, com resultado negativo e cujas cópias apresentaram e entregaram à Autoridade Regional de Saúde, à chegada ao aeroporto, em Ponta Delgada.

2. No dia 07/08/2020 e já durante a estada na ilha de São Miguel, as requerentes AH___ e NK___ realizaram um segundo teste ao COVID19.

3.No dia 10/08/2020 e também já durante a estada na ilha de São Miguel, os requerentes SH___ e SWH___ realizaram um segundo teste ao COVID19.

4.No dia 08/08/2020 a requerente AH___foi, por telefone, informada que o seu teste realizado no dia anterior tinha acusado “detectado”.

5.A partir desse dia 08/08/2020 a requerente AH___ deixou de coabitar com os restantes três requerentes, tendo sempre mantido uma distância nunca inferior a 2 (dois) metros dos mesmos.

6.No dia 10/08/2020 os requerentes SH___, SWH___ e NK___foram, por telefone, informados que os seus testes tinham acusado “negativo”.

7.No dia 10/08/2020 foi a todos os requerentes remetido, por via e-mail, o documento junta a fls. 25, 25verso, 26 e 26 verso, assinado pelo Delegado de Saúde do concelho da Lagoa, em exercício de funções, Dr. Magno José Viveiros Silva, denominado Notificação de Isolamento Profiláctico – Coronavírus SARS- CoV-2/Doença COVID – 19, e dois anexos (apenas um deles em língua inglesa) e no qual se lê (teor igual à excepção da identificação de cada um dos ora Requerentes):

“Isolation (...)

Notificação de

Isolamento Profilático

Coronavírus SARS- CoV-2/Doença COVID – 19

Mário Viveiros Silva Autoridade de Saúde de Lagoa

Nos termos das Circulares Normativas n.s DRSCINF/2020/22 de 2020/03/25 e DRS CNORM2020/39B de 2020/08/04 da AUTORIDADE REGIONAL DE SAÚDE (em anexo) e da Norma n.º 015/2020, de 24/07/2020 da Direcção Geral de Saúde (em anexo) determino o

ISOLAMENTO PROFILÁCTICO

DE

(...)

Portador do Cartão de Cidadão/PASSAPORTE N.º (...), com validade ... até ...com o número de identificação de segurança social pelo período de 08/08/2020 a 22/08/2020 por motivo de perigo de contágio e como medida de contenção de COVID 19 (SARS-Cov-2)

Data 2020/08/10 (...)

8.Os Requerentes solicitaram que lhe enviassem os ditos resultados, tendo sido remetido o relatório do teste feito às Requerentes AH___ e NK___por via e-mail no dia

13/08/2020 e aos Requerentes SH___ e SWH___ no dia de ontem, 24/08/2020, por via e-mail, relatórios estes redigidos em língua portuguesa.
9.Entre os dias 01 e 14 de agosto os requerentes estiveram acomodados no alojamento Marina Mar II, em Vila Franca do Campo.
10.De 14 de agosto em diante os requerentes estão acomodados no "THE LINCE AZORES GREAT HOTEL, CONFERENCE & SPA", em Ponta Delgada (onde actualmente se encontram), por ordem do Delegado de Saúde nos termos descritos em 7 do seguinte modo:
- No quarto 502 encontram-se os requerentes SH___ e SWH___.
- No quarto 501 encontra-se a requerente AH___.
- No quarto 506 encontra-se a requerente NK___.
11.Os requerentes tentaram pelo menos por 3 vezes contactar a linha de apoio telefónico que conhecem (296 249 220) para serem esclarecidos na sua língua ou, ao menos, na língua inglesa, mas nunca tiveram qualquer sucesso, uma vez que apenas atendem e respondem na língua portuguesa, que os requerentes não entendem.
12.No hotel, as refeições são entregues no quarto, pelos serviços do hotel, a horas pré-determinadas e de acordo com uma escolha feita por terceiros, a não ser durante os primeiros 3 dias no Hotel Lynce em que foram servidos pequenos-almoços e as restantes refeições através de room service.
13.No dia 15 de agosto, enquanto cumpria o isolamento profiláctico determinado pelo Delegado de Saúde, a requerente AH___ passou a padecer de uma inflamação na boca, aparentemente resultante do aparelho dentário que usa.
14.Tendo, pelo telefone, para o número 296 249 220, partilhado essa situação com a Autoridade Regional de Saúde, a quem solicitou o necessário suporte médico.
15.Este pedido foi ignorado pela referida linha de apoio, que não proporcionou à requerida AH___ o necessário apoio.
16.Não vislumbrando qualquer apoio, dois dias mais tarde, a 17 de agosto, devidamente protegida por máscara e luvas, a requerente SWH___ saiu do seu quarto, dirigiu-se à farmácia mais próxima do hotel, onde adquiriu uma pomada para debelar temporariamente a situação referida, tendo regressado imediatamente ao hotel e ao seu quarto.
17.No dia 19/08/2020 foi remetido pelo Delegado de Saúde, Dr. JMS___, aos Requerentes e-mail, onde nomeadamente se lê:
"(...) A AH___ só é dada como curada após ter um teste negativo e um 2.º teste de cura negativo, quando isso acontecer a delegação de saúde entrará em contacto (...) (sic).
18.No dia 21/08/2020 foi transmitido aos quatro requerentes, pelo Delegado de Saúde Dr. JMS___, por via de correio electrónico a seguinte mensagem: "A saber, quando acabarem a quarentena têm de fazer teste e se este for negativo podem sair de casa" (sic).
19.Nesse mesmo dia 21 de agosto o requerente SH___ questionou o referido médico e Delegado de Saúde, Dr. JMS___, por mensagem de correio electrónico que remeteu, o seguinte (traduzido para a língua portuguesa em regime livre):
"Caro Dr. JMS___,
Já fizemos dois testes COVID / pessoa, todos foram negativos (SH___, SWH___, NK___).
..e depois disso passamos 2 semanas em isolamento, e nenhum de nós acusa qualquer sintoma!!

Temos os documentos do Dr. MMS___, confirme.
Ninguém nos disse alguma coisa sobre os novos testes após o tempo de isolamento?!
Já remarcamos os nossos voos e planeamos deixar a ilha.
Explique o motivo da sua declaração.
Por que não foi feito ontem o teste COVID de AH___?
Saudações,
SH___"
20.Os requerentes não receberam qualquer resposta a esta mensagem de correio electrónico, à excepção da Requerente AH___ a quem foi comunicado agendamento de realização de novo teste de despiste, em concreto, para o próximo dia 29/08/2020.
21.No dia 20/08/2020 a requerente AH___ realizou um terceiro teste ao COVID19, tendo no dia seguinte (21/08/2020), apenas por telefone, sido informada que o resultado tinha acusado "detectado".
22.A requerente AH___ solicitou que lhe enviassem uma evidência escrita desse resultado positivo, o que lhe foi enviado por via e-mail no dia de ontem, dia 24/08/2020.
23.Os Requerentes questionaram os funcionários da recepção do hotel onde se encontram, tendo-lhes sido dito que nenhum dos quatro requerentes, sem excepção, poderá ausentar-se dos quartos.
24.Os requerentes não apresentam, nem nunca apresentaram, qualquer sintoma da doença (febre, tosse, dores musculares, espirros, falta de olfacto ou palato).
25.Aos requerentes não foi explicado o conteúdo dos dois documentos que lhe foram remetidos com os escritos elencados no ponto 7.
26.Os requerentes têm residência habitual na República Federal da Alemanha, identificada nestes autos.

Fundamentação:

A questão que aqui se coloca, assente que os Requerentes se encontram privados da sua liberdade (desde o passado dia 10 de agosto até à presente data, conforme decorre dos factos provados) e, consequentemente, podendo socorrer-se do presente instituto do habeas corpus - como passaremos a expor –, reconduz-se a saber se existe ou não fundamento legal para esta privação da liberdade.

Com efeito, sem questionar sequer a constitucionalidade orgânica da Resolução do Conselho do Governo Regional nº 207/2020, de 31 de Julho de 2020, actualmente em vigor no âmbito dos procedimentos aprovados pelo Governo dos Açores na contenção da disseminação do vírus SARS-COV-2 nesta Região Autónoma, na situação em apreço a detenção/confinamento dos Requerentes desde o passado dia 10 de agosto encontra-se materializada por uma comunicação realizada por via e-mail, em língua portuguesa, nos termos dados como provados sob o ponto 7.

Ora, conforme resulta do referido ponto 7 dos factos provados, a autoridade de saúde regional, por meio do respectivo Delegado de Saúde da área territorial onde os Requerentes se encontravam hospedados, determinou o isolamento profiláctico destes ao abrigo das Circulares Normativas n.s DRSCINF/2020/22 de 2020/03/2025 e DRS CNORM2020/39B de 2020/08/04 da AUTORIDADE REGIONAL DE SAÚDE e da Norma n.º 015/2020, de 24/07/2020 da Direcção Geral de Saúde. E, foi através de uma comunicação com a aludida sustentação, saliente-se, em circulares normativas e uma norma da Direcção Geral de Saúde, que a Autoridade Regional de Saúde privou os Requerentes da sua liberdade, porquanto dos factos provados deriva à saciedade que *estes, no rigor dos conceitos, estiveram detidos do dia 10 ao dia 14 de Agosto de 2020 num empreendimento hoteleiro em Vila Franca do Campo e do dia 14 de agosto de 2020 até à presente data confinados, e portanto detidos, em quarto de hotel nesta cidade de Ponta Delgada. Não podemos olvidar, até porque sobressai do elenco dos factos provados, que o poder de circulação e direito de mobilidade dos Requerentes – ou de qualquer outro individuo que se encontre em idêntica situação - estão de tal modo limitados que a primeira saída dos quartos onde se encontram foi para se deslocarem a este tribunal e prestar declarações (com excepção da deslocação à farmácia da Requerente SWH___ em claro desespero para acudir às dores da sua filha nos termos provados).*

Em suma, analisada a factualidade apurada é inexorável concluir que estamos perante uma verdadeira privação da liberdade pessoal e física dos requerentes, não consentida pelos mesmos, que os impede não só de se deslocar, como de estar em família, vivendo há cerca de 16 dias separados (os requerentes SH___ e SWH___ e a sua filha, aqui Requerente, AH___) e, no caso da Requerente NK___ totalmente sozinha, sem qualquer contacto físico seja com quem for. Dizer que não há privação da liberdade porque a qualquer momento podem ausentar-se dos respectivos quartos, em que se encontram é uma falácia, bastando atentar às comunicações que lhes foram efectuadas após o dia 10 de agosto, nenhuma delas na língua alemã, e as condições em que têm vivido (não se descurando que se tratam de cidadãos estrangeiros com a barreira linguística inerente) ou solicitar o seu regresso ao local de origem é uma falácia, e, para tal conclusão basta atentar às últimas comunicações efectuadas em português, sublinhe-se da qual se destaca a dada como provada sob o ponto 8, em concreto "A saber, quando acabarem a quarentena têm de fazer teste e se este for negativo podem sair de leia-se casa como o hotel onde se encontram confinados em 3 quartos.

Logo, encontrando-se os Requerentes privados da sua liberdade, perante as circunstâncias provadas, cumpre traçar o caminho em que nos movemos, iniciando o percurso pelo farol norteador do sistema legislativo português: a Constituição da República Portuguesa.

Assim, no plano da hierarquia das normas, impõe-se relembrar que, conforme dispõe o artigo 1º da CRP, "Portugal é uma República soberana, baseada na dignidade da pessoa humana e na vontade popular e empenhada na construção de uma sociedade livre, justa e solidária.". Daí se retira, de modo inequívoco, que a unidade de sentido em que radica o nosso sistema de direitos fundamentais se estriba na dignidade humana – o princípio da dignidade da pessoa humana é a referência axial de todo o sistema de direitos fundamentais.

Um deles, dos mais relevantes atenta a sua natureza estruturante do próprio estado democrático, é o princípio da igualdade, previsto no artigo 13º, da CRP, onde se dispõe, no seu nº 1, que "Todos os cidadãos têm a mesma dignidade social e são iguais perante a lei.", acrescentando o nº 2, que "Ninguém pode ser privilegiado, beneficiado, prejudicado, privado de qualquer direito ou isento de qualquer dever em razão de ascendência, sexo, raça, língua, território de origem, religião, convicções políticas ou ideológicas, instrução, situação económica, condição social ou orientação sexual.".

E, no que aqui importa particularmente, sob a epígrafe "direito à liberdade e à segurança" dispõe o artigo 27º, nº 1, da CRP, "Todos têm direito à liberdade e à segurança", referindo José Lobo Moutinho, em anotação a tal artigo, que "A liberdade é

um momento absolutamente decisivo e essencial - para não dizer, o próprio e constitutivo modo de ser – da pessoa humana (Ac. n° 607/03: "exigência ôntica"), que lhe empresta aquela dignidade em que encontra o seu fundamento granítico a ordem jurídica (e, antes de mais, jurídico-constitucional) portuguesa (artigo 1º da Constituição). Pode dizer-se, nesse sentido, a pedra angular do edifício social" (Ac. n° 1166/96)" (aut.cit., in op. Cit., pág. 637).
*Não sendo a liberdade humana unidimensional, podendo assumir múltiplas dimensões, do que são exemplo os artigos 37º e 41º, da CRP, a liberdade em causa no artigo 27º, é a liberdade física, entendida como liberdade de movimento corpóreo, de ir e vir, a liberdade ambulatória ou de locomoção, prevendo-se no nº 2 deste último artigo que "**Ninguém pode ser total ou parcialmente privado da liberdade, a não ser em consequência de sentença judicial condenatória pela prática de acto punido por lei com pena de prisão ou de aplicação judicial de medida de segurança." – nosso sublinhado.***
As excepções a tal princípio encontram-se tipificadas no nº 3, o qual dispõe que:
*"**Exceptua-se deste princípio a privação da liberdade, pelo tempo e nas condições que a lei determinar, nos casos seguintes:***
a)Detenção em flagrante delito;
b)Detenção ou prisão preventiva por fortes indícios de prática de crime doloso a que corresponda pena de prisão cujo limite máximo seja superior a três anos;
c)Prisão, detenção ou outra medida coactiva sujeita a controlo judicial, de pessoa que tenha penetrado ou permaneça irregularmente no território nacional ou contra a qual esteja em curso processo de extradição ou de expulsão;
d)Prisão disciplinar imposta a militares, com garantia de recurso para o tribunal competente;
e)Sujeição de um menor a medidas de protecção, assistência ou educação em estabelecimento adequado, decretadas pelo tribunal judicial competente;
f)Detenção por decisão judicial em virtude de desobediência a decisão tomada por um tribunal ou para assegurar a comparência perante autoridade judiciária competente;
g)Detenção de suspeitos, para efeitos de identificação, nos casos e pelo tempo estritamente necessários;
h)Internamento de portador de anomalia psíquica em estabelecimento terapêutico adequado, decretado ou confirmado por autoridade judicial competente."
Por fim, cumpre relembrar que, havendo privação da liberdade contra o disposto na Constituição e na Lei, o Estado fica constituído no dever de indemnizar o lesado nos termos que a lei estabelecer, conforme decorre do nº 5 do artigo 27º, salientando-se que, em consonância com o art.º 3.º da CRP:
(...) 2. O Estado subordina-se à Constituição e funda-se na legalidade democrática.
3. A validade das leis e dos demais actos do Estado, das regiões autónomas, do poder local e de quaisquer outras entidades públicas depende da sua conformidade com a Constituição.
Aqui chegados, traçada o território legal, vejamos de perto o quadro em que se moveu a Autoridade Regional de Saúde na situação em análise.
Os Requerentes SH__SWH__ e NK_ realizaram teste de despiste ao vírus SARS-CoV-2 cujo resultado para todos foi negativo, tendo resultado o mesmo teste positivo para a Requerente AH___, o que conduziu à citada ordem de isolamento profiláctico e consequente permanência destes nos termos expostos e provados.

Pelo que, perante o teor da notificação efectuada aos Requerentes este tribunal não pode deixar de expressar, ab initio, a sua perplexidade perante a determinação de isolamento profiláctico aos quatro Requerentes.
Conforme decorre da definição dada pela Direcção Geral da Saúde, "A quarentena e o isolamento, são medidas de afastamento social essenciais em saúde pública. São especialmente utilizadas em resposta a uma epidemia e pretendem proteger a população da transmissão entre pessoas. A diferença entre a quarentena e o isolamento parte do estado de doença da pessoa que se quer em afastamento social. Ou seja:
"quarentena é utilizada em pessoas que se pressupõe serem saudáveis, mas possam ter estado em contacto com um doente infectado;
isolamento é a medida utilizada em pessoas doentes, para que através do afastamento social não contagiem outros cidadãos." (em https://www.sns24.gov.pt/tema/doencas-infecciosas/covid-19/isolamento/?fbclid=IwAR34hD77oLCpxUVYJ9Ol4ttgwo4tsTOvPfIa3Uyoh0EJEbCs3jEihkaEPAY#sec-0).
Volvendo ao caso vertente, a Autoridade Regional de Saúde decidiu fazer tábua rasa de conceitos essenciais, porque delimitam o tratamento diferenciado (porque distinto, passe o pleonasmo), das situações de pessoas contagiadas e das que com esta estiveram em contacto, perante a ordem de isolamento profiláctico a todos os requerentes, apesar de apenas um deles ter resultados positivos ao aludido teste de despiste. E, mais decidiu, fazer letra morta da própria Resolução do Conselho do Governo n.º 207/2020 de 31 de Julho, imiscuindo-se à submissão obrigatória a validação judicial do tribunal competente decretada que seja quarentena obrigatória, quando deriva à saciedade dos factos provados que os Requerentes SH__SWH__ e NK___, quanto muito, estão sujeitos a quarentena obrigatória.
Não o fez nas 24 horas previstas no ponto 6 da citada Resolução, nem sequer em prazo mais lato - como nas 48 horas previstas no artigo 254.º, n.º 1, alínea a), do Código de Processo Penal, ou no artigo 26.º, n.º 2, da LSM – continuando por efectuar qualquer comunicação e, por esta via, a restrição evidente da liberdade dos Requerentes SH__SWH__ e NK_ sempre será ilegal.
Neste passo, a citada Resolução do Conselho do Governo n.º 207/2020, de 31 de julho de 2020, prevê no seu ponto 4 que nos casos do resultado do teste ao vírus ao SARS-CoV-2 ser positivo, a autoridade de saúde local, no âmbito das suas competências, determinará os procedimentos a seguir. A Requerente AH___ positiva no teste de despiste do vírus em causa, foi notificada, reitere-se nos mesmos termos que os demais Requerentes, da ordem de isolamento profiláctico entre os dias 10/08/2020 a 22/08/2020.
Neste ponto, impõe-se deixar claro que a notificação efectuada conforme provado sob o ponto 7, é trazida daquilo que consta da Norma da DGS015/2020, regra a que se alude na mesma para além das circulares normativas (disponível para consulta em https://www.dgs.pt/directrizes-da-dgs/normas-e-circulares-normativas/norma-n-0152020-de-24072020-pdf.aspx),e nos diz, no que aqui interessa: (...) Contactos com Exposição de Alto Risco
15. Um contacto classificado como tendo exposição de alto risco, nos termos do Anexo 1 fica sujeito a:
a.Vigilância activa durante 14 dias, desde a data da última exposição;
b.Determinação de isolamento profiláctico, no domicílio ou outro local definido a nível

local, pela Autoridade de Saúde, até ao final do período de vigilância activa, de acordo com o modelo dos Despachos n.º 2836-A/2020 e/ou n.º 3103-A/20202 (modelo acessível em http://www.seg-social.pt/documents/10152/16819997/GIT_70.docx/e6940795-8bd0-4fad-b850-ce9e05d80283)
Seguindo esta norma da Direcção Geral de Saúde, lê-se, entre o demais, na circular normativa n.º DRSCNORM/2020/39B, de 2020-08-04 (disponível para consulta em http://www.azores.gov.pt/NR/rdonlyres/25F80DC1-51E6-4447-8A38-19529975760/1125135/CN39B_signed1.pdf),
(...)
a.Contactos próximos de alto risco
Os contactos próximos de alto risco são tratados como casos suspeitos até ao resultado laboratorial do caso suspeito. Estes contactos próximos devem fazer rastreio para SARS-CoV-2. São considerados contactos de alto risco: i. Coabitação com caso confirmado de COVID-19; (...)
ii. Vigilância e Controlo de Contactos Próximos
3. Os contactos próximos de alto risco, atendendo a que, actualmente, se estima que o período de incubação da doença (tempo decorrido desde a exposição ao vírus até ao aparecimento de sintomas) seja entre 1 e 14 dias, os mesmos deverão cumprir 14 dias de isolamento profiláctico, mesmo que apresentem testes de despiste negativos durante esse período, devendo ser realizado teste ao 14º dia. Caso o resultado do teste do 14º dia seja negativo, têm alta. Caso os contactos próximos de alto risco coabitem com o caso positivo, apenas deverão ter alta aquando da determinação da cura do caso positivo, devendo, por esta via, ser prorrogado o respectivo isolamento profiláctico.
(...)
13. Cumprimento de isolamento profiláctico
Todas as pessoas identificadas como caso suspeito, até serem conhecidos os resultados negativos, cumprem isolamento profiláctico;
Todas as pessoas que testaram positivo para Covid-19 e que têm alta após teste de cura (internamento ou domicílio), não precisam efectuar novo período de isolamento de 14 dias nem repetir novo teste ao 14º dia.
Todos os passageiros que desembarquem nos aeroportos da Região provenientes dos aeroportos localizados em zonas consideradas como sendo zonas de transmissão comunitária activa ou com cadeias de transmissão activas do vírus SARS-CoV-2 devem cumprir os procedimentos em vigor na Região à data.
Aqui chegados, analisemos o valor jurídico de normas/orientações da Direcção Geral de Saúde e da circular normativa 39B, de04/08/2020, da Direcção Regional de Saúde, *não restando dúvidas que entramos na esfera das orientações administrativas.*
A este propósito, com a especificidade de se reportar à Autoridade Tributária – a qual tem a mesma posição jurídica administrativa da Autoridade Nacional de Saúde no ius imperium do Estado-, CASALTA NABAIS (Direito Fiscal, 6.ª ed., Almedina, pág. 197), " as chamadas orientações administrativas, tradicionalmente apresentadas nas mais diversas formas como instruções, circulares, ofícios-circulares, ofícios-circulados, despachos normativos, regulamentos, pareceres, etc.", que são muito frequentes no direito fiscal constituem "regulamentos internos que, por terem como destinatário apenas a administração tributária, só esta lhes deve obediência, sendo, pois, obrigatórios apenas para os órgãos situados hierarquicamente abaixo do órgão autor dos mesmos.

Por isso não são vinculativos nem para os particulares nem para os tribunais. E isto quer sejam regulamentos organizatórios, que definem regras aplicáveis ao funcionamento interno da administração tributária, criando métodos de trabalho ou modos de actuação, quer sejam regulamentos interpretativos, que procedem à interpretação de preceitos legais (ou regulamentares).
É certo que eles densificam, explicitam ou desenvolvem os preceitos legais, definindo previamente o conteúdo dos actos a praticar pela administração aquando da sua aplicação. Mas isso não os converte em padrão de validade dos actos que suportam. Na verdade, a aferição da legalidade dos actos da administração tributária deve ser efectuada através do confronto directo com a correspondente norma legal e não com o regulamento interno, que se interpôs entre a norma e o acto".
Ora, o problema da relevância normativa das Circulares da Administração (Tributária) foi já colocado e apreciado nos Acórdãos do Tribunal Constitucional nº 583/2009 e 42/14, de 18.11.2009 e de 09.012.2014, respectivamente, tendo aquele Tribunal decidido, com o que concordamos, que as prescrições contidas nas Circulares da Administração Tributária, independentemente da sua irradiação persuasiva na prática dos cidadãos, não constituem normas para efeitos do sistema de controlo de constitucionalidade cometido ao Tribunal Constitucional.
Como se sublinhou naquele aresto (Acórdão 583/2009) "(...) Esses actos, em que avultam as "circulares", emanam do poder de auto-organização e do poder hierárquico da Administração. Contêm ordens genéricas de serviço e é por isso e só no respectivo âmbito subjectivo (da relação hierárquica) que têm observância assegurada. Incorporam directrizes de acção futura, transmitidas por escrito a todos os subalternos da autoridade administrativa que as emitiu. São modos de decisão padronizada, assumidos para racionalizar e simplificar o funcionamento dos serviços. Vale isto por dizer que, embora indirectamente possam proteger a segurança jurídica e assegurar igualdade de tratamento mediante aplicação uniforme da lei, não regulam a matéria sobre que versam em confronto com os particulares, nem constituem regra de decisão para os tribunais."
Consequentemente, faltando-lhes força vinculativa heterónoma para os particulares e não se impondo ao juiz senão pelo valor doutrinário que porventura possuam, as prescrições contidas nas "circulares" não constituem normas para efeitos do sistema de controlo de constitucionalidade da competência do Tribunal Constitucional.
O que fica dito, permite-nos concluir que as orientações administrativas veiculadas sob a forma de circular normativa, como no presente caso, não constituem disposições de valor legislativo que possam ser objecto de declaração de inconstitucionalidade formal - vide Acórdão do Supremo Tribunal Administrativo, de 21/06/2017, disponível para consulta em www.dgsi.pt.
***E, isto para deixar claro que os normativos invocados pela Autoridade Regional de Saúde que sustentaram a privação de liberdade imposta aos Requerentes por meio de notificação de isolamento profiláctico tratam-se de orientações administrativas não vinculativas para os Requerentes.** Aliás. basta atentar a quem são dirigidas respectivamente:*
Circular Normativa n.º DRSCNORM/2020/39B:"Para: Unidades de Saúde do Serviço Regional de Saúde, Delegados de Saúde Concelhios (C/c Serviço Regional de Protecção Civil e Bombeiros dos Açores, Linha de Saúde Açores) Assunto: Rastreios a SRAS-CoV-2 e abordagem dos casos suspeitos ou confirmados de infecção por SARS-CoV-2 Fonte:

Direcção Regional da Saúde (...)
Norma 015/2020, de 24/07/2020: "ASSUNTO: COVID-19: Rastreio de Contactos
PALAVRAS-CHAVE: Coronavírus, SARS-CoV-2, COVID-19, Rastreio de Contactos (Contact Tracing), Investigação Epidemiológica
PARA: Sistema de Saúde (...).
Nesta sequência, e, em jeito de síntese, não pode este tribunal deixar de sublinhar que o presente caso, permitimo-nos dizer aberrante, de privação de liberdade de pessoas, carece em absoluto de qualquer fundamento legal, e não se venha novamente com o argumento de que está em causa a defesa da saúde pública porque o tribunal age sempre do mesmo modo, ou seja, em conformidade com a lei, aliás, daí a necessidade de confirmação judicial consagrada na Lei de Saúde Mental no caso de internamento compulsivo, porquanto da factualidade apurada e do exposto resulta:
- Os Requerentes encontram-se confinados ao espaço de um quarto há cerca de 16 dias, com base numa notificação de "isolamento profiláctico" até ao dia 22/08/2020, período que já foi ultrapassado e a notificação operada, que de qualquer modo é ilegal como meio de detenção de pessoas pelas razões já explicitadas (bastando atentar às normas constitucionais supra expostas), caducou;
- Aos Requerentes nunca foi transmitida qualquer informação, comunicação, notificação, como devido, na sua língua materna, nem lhes foi disponibilizado intérprete, desde logo em flagrante violação da Convenção Europeia dos Direitos do Homem (art.ºs 5.º, n.º 2 e 6.º, n.º 3, al. a) e das normas processuais penais (cfr. art.º 92.º do Código Processo Penal), ou seja, no nosso sistema jurídico detida uma pessoa estrangeira e sem domínio da língua portuguesa é nomeado de imediato intérprete, e, no caso dos Requerentes que se limitaram a viajar para esta ilha e usufruir da sua beleza, nunca lhes foi concedida tal possibilidade;
- Os Requerentes após o dia 22/08/2020 encontram-se confinados ao espaço de um quarto com base nas seguintes comunicações:
- No dia 19/08/2020 foi remetido pelo Delegado de Saúde, Dr. JMS___, aos Requerentes e-mail, onde nomeadamente se lê:
"(...) A AH___ só é dada como curada após ter um teste negativo e um 2.º teste de cura negativo, quando isso acontecer a delegação de saúde entrará em contacto (...) (sic).
- No dia 21/08/2020 foi transmitido aos quatro requerentes, pelo Delegado de Saúde Dr. JMS___, por via de correio electrónico a seguinte mensagem: "A saber, quando acabarem a quarentena têm de fazer teste e se este for negativo podem sair de casa" (sic);
- A privação de liberdade dos Requerentes não foi sujeita a qualquer crivo judicial.
Como dissemos inicialmente, podíamos, ainda, equacionar a constitucionalidade orgânica da Resolução do Conselho do Governo nº 1207/2020, de 31 de Junho, contudo, cremos ser questão despicienda para o objecto da decisão a proferir, que se quer célere, pois mesmo à luz de tal resolução a decisão não pode ser diversa, assente a decisão do Tribunal Constitucional, de 31/07/2020, no âmbito do processo n.º 424/2020, e, porque a posição da Autoridade Regional de Saúde nas presentes circunstâncias reconduz-se à aplicação de circulares normativas, com o valor que explicitamos supra.
Por último, e porque este tribunal tem vindo a pronunciar-se sucessiva e recentemente no âmbito do presente instituto de "habeas corpus" em face das ordens emanadas pela Autoridade Regional de Saúde, permitimo-nos subscrever e sublinhar o seguinte trecho

da primeira decisão deste Juízo de Instrução Criminal:
"A questão do confinamento compulsivo em caso de doenças contagiosas, e os termos em que o mesmo deve ocorrer, é uma questão premente, e que não encontra suporte no artigo 27º, nº 3, da CRP, designadamente na sua alínea h), onde apenas se prevê o internamento de portador de anomalia psíquica em estabelecimento terapêutico adequado, decretado ou confirmado por autoridade judicial competente. Urge legislar sobre tal matéria, estabelecendo-se, de modo claro, os princípios fundamentais a que deve obedecer, deixando os aspectos detalhados para o direito derivado - e somente esses.
Pois, como refere o Professor Gian Luigi Gatta, que aqui citamos numa tradução livre, ***"neste momento, as energias do país estão focadas na emergência. Mas a necessidade de proteger os direitos fundamentais, também e acima de tudo em caso de emergência, exigindo-se aos Tribunais que façam sua parte. Porque, além da medicina e da ciência, também o direito - e o direito dos direitos humanos em primeiro lugar - devem estar na vanguarda: não para proibir e sancionar - como está sendo sublinhado demais nos dias de hoje – mas para garantir e proteger todos nós. Hoje a emergência é chamada de coronavírus. Nós não sabemos o amanhã. E o que fazemos ou não fazemos hoje, para manter a cumprimento dos princípios fundamentais do sistema, pode condicionar nosso futuro."*** *(in "I diritti fondamentali alla prova del coronavirus. Perché è necessaria una legge sulla quarantena",)".*
Não será difícil admitir e aceitar que a turbulência legislativa gerada em torno da contenção da propagação da COVID-19 teve – e continuará a ter – na sua razão de ser a proteção da saúde pública, mas nunca esta turbulência poderá ferir de morte o direito à liberdade e segurança e, em última análise, o direito absoluto à dignidade humana.
Resta decidir em conformidade.
(...)
Pelo que, à luz do supra exposto, por ilegal a detenção dos Requerentes SH__SWH___, AH___ e NK___, decido julgar procedente o presente pedido de habeas corpus e, consequentemente, determino a restituição imediata dos mesmos à liberdade.
2. A ora recorrente formulou as seguintes conclusões, que extraiu da sua motivação:
1.O presente recurso tem por objecto a decisão proferida pelo douto Tribunal a quo entendeu ser "por ilegal a detenção dos Requerentes SH__SWH___, AH___ e NK___" e decidiu "julgar procedente o presente pedido de habeas corpus e, consequentemente, determino a restituição imediata dos mesmos à liberdade.";
2.Apenas por uma questão de economia processual, ou seja, por ser pouco relevante para apreciação do mérito da causa, não se recorre da factualidade dada como provada, não deixando, no entanto, de referir que a mesma se baseou unicamente nas declarações dos próprios requerentes.
3.A decisão recorrida ao invocar que a recorrente não cumpriu com o ponto 6 da Resolução do Conselho do Governo Regional dos Açores n.º 207/2020, de 31 de Julho de 2020, violou o âmbito de aplicação da mesma Resolução, definido no ponto 1 da mesma Resolução;
4.A validação judicial de quarentena obrigatória, prevista no ponto 6 da dita resolução, apenas se aplica a quarentena obrigatória decretada aos passageiros que não aceitem, em alternativa, qualquer um dos procedimentos, previstos no ponto 1 da citada Resolução;
5.Os requerentes cumpriram com o procedimento previsto na alínea a) do ponto 1 da

Resolução ° 207/2020, de 31 de Julho de 2020, pelo que nunca poderiam ser sujeitos à quarentena obrigatória, ao abrigo daquela Resolução e, consequentemente, não há lugar à validação judicial, prevista no ponto 6 da Resolução n.° 207/2020, de 31 de Julho de 2020.

6.Ao contrário do defendido na decisão recorrida, o ordenamento jurídico português permite a adoptação de medidas de excepção, incluindo separação de pessoas, consequente decretamento de confinamento obrigatório de pessoas infectadas e com elevada probabilidade de estar infectadas, através do mecanismo previsto no artigo 17.° da Lei n.° 81/2009, de 21 de agosto;

7.O Conselho de Ministros legitimamente fez uso do poder regulamentar excepcional, previsto no artigo 17.° da Lei n.° 81/2009, através das Resoluções do Conselho de Ministros n.° 55-A/2020, de 31 de julho de 2020 e n.° 63-A/2020, de 14 de agosto;

8.O n.° 2 da Resolução do Conselho de Ministros n.° 55-A/2020, de 31 de Julho de 2020, mandou aplicar a todo o território nacional medidas de carácter excepcional, necessárias ao combate à COVID -19, designadamente as previstos no regime anexo à aquela resolução;

9.O artigo 2.° do Anexo decretou que:

"Artigo 2.º

Confinamento obrigatório

1 — Ficam em confinamento obrigatório, em estabelecimento de saúde, no respectivo domicílio ou noutro local definido pelas autoridades de saúde:

a)Os doentes com COVID -19 e os infectados com SARS -CoV-2;

b)Os cidadãos relativamente a quem a autoridade de saúde ou outros profissionais de saúde tenham determinado a vigilância activa.

2 – (...)"

10. A requerente AH___ ao estar infectada com o vírus SARS-CoV-2, em cumprimento do artigo 2.º, n.º 1, alínea a) do Anexo I da Resolução do Conselho de Ministro n.º 55-A/2020, tinha de estar em confinamento obrigatório;

11. O Tribunal a quo ao decretar o habeas corpus de AH___ e permitir a sua livre circulação violou o artigo 17.° da Lei n.° 81/2009, de 21 de agosto, por referência ao artigo 2.º, n.º 1, alínea a) do Anexo I da Resolução do Conselho de Ministro n.º 55-A/2020;

12. Os requerentes SH__SWH__ e NK_ de acordo com as regras estipuladas pela Autoridade Nacional de Saúde, constantes da Norma 015/2020, de 24/07/2020, são contactos com Exposição de Alto Risco, devendo ficar sujeitos a:

a.Vigilância activa durante 14 dias, desde a data da última exposição;

b.Determinação de isolamento profiláctico, no domicílio ou outro local definido a nível local, pela Autoridade de Saúde, até ao final do período de vigilância activa, de acordo com o modelo dos Despachos n.° 2836-A/2020 e/ou n.° 3103-A/20202"

13. Os requerentes SH__SWH__ e NK_ ao estarem sujeitos a vigilância activa, em cumprimento do artigo 2.°, n.° 1, alínea b) do Anexo I da Resolução do Conselho de Ministro n.° 55-A/2020, tinham de estar em confinamento obrigatório;

14. O Tribunal a quo ao decretar o habeas corpus de SH__SWH__ e NK_ e permitir a sua livre circulação violou o artigo 17.° da Lei n.° 81/2009, de 21 de agosto, por referência ao artigo 2.º, n.º 1, alínea b) do Anexo I da Resolução do Conselho de Ministro n.º 55-A/2020.

15. Impõe-se que a decisão recorrida seja revogada e substituída por outro que, valide o confinamento obrigatório dos requerentes, por serem portadores do vírus SARS -CoV-2 (AH___) e por estarem em vigilância activa, por exposição de alto risco, decretada pelas autoridades de saúde (SH__SWH__ e NK___).

3. Na sua resposta, o MºPº extraiu as seguintes conclusões:

1 — O acórdão do Tribunal Constitucional de 31-07-2020 (Proc. 403/2020;1.' Secção; Cons. José António Teles Pereira), depois de concluir que o confinamento obrigatório, seja através de quarentena seja através de isolamento profiláctico, constitui uma verdadeira privação da liberdade não prevista no art. 27.°, n.° 2, da C.R.P., e que todas as privações da liberdade carecem de autorização prévia da Assembleia da República, *o que não foi o caso das Resoluções do Governo Regional dos Açores que impuseram uma quarentena obrigatória, considerou verificada a inconstitucionalidade orgânica das normas referidas.*

2 — Estas normas, declaradas inconstitucionais pelo Tribunal Constitucional, são em tudo materialmente idênticas às que constam das Resoluções do Conselho de Ministros n.°s 55-A/2020, de 31-07, 63-A/2020, de 14-08, e 70-A/2020, de 11-09, e n.° 88-A/2020, de 14-10, na medida em que prevêem privações da liberdade não previstas em diploma legal adequado e emanado da entidade competente, bem como não se encontram nas excepções previstas no art. 27.°, n.° 3, da C.R.P., pelo que estas também devem ser desaplicadas por violação do art. 27.°, n.° 1, da C.R.P..

3 — Prevendo o art. 5.°, n.° 1, al. e), da Convenção Europeia dos Direitos do Homem (Convenção para a Protecção dos Direitos do Homem e das Liberdades Fundamentais — Roma, 04-11-1950), relativo ao Direito à liberdade e à segurança, que «Toda a pessoa tem direito à liberdade e segurança» e que «Ninguém pode ser privado da sua liberdade, salvo nos casos seguintes e de acordo com o procedimento legal: (...) «Se se tratar da detenção legal de uma pessoa susceptível de propagar uma doença contagiosa, de um alienado mental, de um alcoólico, de um toxicómano ou de um vagabundo», podemos concluir que a privação da liberdade de uma pessoa susceptível de propagar uma doença contagiosa é uma forma de detenção e que, de acordo com a Convenção, é possível os Estados preverem na sua legislação interna a detenção destas pessoas.

4 — Tendo em conta o princípio constitucional da tipicidade das medidas privativas da liberdade, e não prevendo o art. 27.°, da C.R.P., em nenhuma das alíneas do seu número 3, a privação da liberdade de uma pessoa "susceptível de propagar uma doença contagiosa",

5 — E tendo a alínea h) — que prevê o internamento de portador de anomalia psíquica em estabelecimento terapêutico adequado —sido acrescentada pelo art. 11.0, n.° 6, da Lei Constitucional n.° 1/97, de 20 de Setembro (4.' revisão constitucional), numa altura em que a Convenção Europeia dos Direitos do Homem já previa expressamente a detenção de pessoa susceptível de propagar doença contagiosa,

6 — E que o legislador constitucional, nem na referida revisão constitucional nem noutra posterior, acrescentou outra alínea ao n.° 3 do art. 27.° a prever esta possibilidade, como fez relativamente ao internamento de portador de anomalia psíquica, podemos concluir que estamos perante uma decisão consciente do legislador constitucional em não permitir que se proceda à privação da liberdade de pessoa susceptível de propagar doença contagiosa, apenas por esse facto.

7 — Da análise do regime constitucional do direito à liberdade e à segurança previsto no

art. 27.°, n.° 1, da C.R.P., podemos concluir, assim, que não é possível o legislador, ainda que através da Assembleia da República ou do Governo por esta autorizado, criar privações da liberdade que não estejam previstas no n.° 3 do referido normativo constitucional, nomeadamente no que respeita a pessoas portadoras de doenças infecto-contagiosas, sejam estas privações da liberdade confinamentos, quarentenas ou isolamentos profilácticos, sem incorrerem as eventuais normas criadas para esse efeito numa inconstitucionalidade material por violação do referido normativo constitucional.
8 — Volvendo agora ao regime legal do internamento de portadores de doenças contagiosas, a Lei n.° 2036 de 09-08-1949 previa a possibilidade de promover o isolamento ou o internamento de pessoas portadoras de doenças infecto-contagiosas, mas apenas, neste último caso, nas situações em que se verificava grave perigo de contágio, havendo recurso para uma autoridade da decisão de isolamento ou internamento.
9 — Por sua vez, o art. 17.° da Lei n.° 81/2009, de 21-08, que revogou a Lei n.° 2036 de 09¬08-1949, permite ao membro do Governo responsável pela área da saúde um poder regulamentar especial, de acordo com o estipulado pela base XX da Lei n.° 48/90, de 24-08 (Lei de Bases da Saúde), nomeadamente, «tomar medidas de excepção indispensáveis em caso de emergência em saúde pública, incluindo a restrição, a suspensão ou o encerramento de actividades ou a separação de pessoas que não estejam doentes, meios de transporte ou mercadorias, que tenham sido expostos, de forma a evitar a eventual disseminação da infecção ou contaminação».
10 ***— Daqui se retira, desde logo, que não está prevista na presente lei, como estava previsto na Lei n.° 2036 de 09-08-1949, a possibilidade de promover o isolamento ou o internamento de pessoas portadoras de doenças infecto-contagiosas.*** *Por outro lado, devendo as medidas tomadas pelas autoridades de saúde respeitar a Constituição e a lei e não prevendo a Lei Constitucional a privação da liberdade das pessoas portadoras de doenças infecto-contagiosas, a interpretação a dar à expressão «separação de pessoas que não estejam doentes, meios de transporte ou mercadorias, que tenham sido expostos», para estar de acordo com a Constituição da República Portuguesa não pode atingir o núcleo do direito à liberdade, ou seja, não devem constituir uma privação total da liberdade.*
11 — Por outro lado, a actual Lei de Bases da Saúde — Lei n.° 95/2019, de 04-09 — prevê na Base 34, relativa à defesa da saúde pública, que a autoridade de saúde pública pode «b) Desencadear, de acordo com a Constituição e a lei, o internamento ou a prestação compulsiva de cuidados de saúde a pessoas que, de outro modo, constituam perigo para a saúde pública».
12 — Também a Lei n.° 82/2009, de 02-04, que regulamenta o regime jurídico da designação, competência e funcionamento das entidades que exercem o poder de autoridades de saúde, prevê no seu art. 5.° as competências da autoridade de saúde, nomeadamente, «c) Desencadear, de acordo com a Constituição e a lei, o internamento ou a prestação compulsiva de cuidados de saúde a indivíduos em situação de prejudicarem a saúde pública».
13 — Daqui se retira que, devendo as medidas tomadas pelas autoridades de saúde respeitar a Constituição e a lei, e não prevendo a Lei Constitucional a privação da liberdade das pessoas portadoras de doenças infecto-contagiosas, caso a interpretação a dar à expressão «internamento ou a prestação compulsiva de cuidados de saúde a

indivíduos em situação de prejudicarem a saúde pública» seja no sentido de que as autoridades de saúde podem ordenar o internamento, ou outra medida restritiva da liberdade de circulação, ou a prestação compulsiva de cuidados de saúde de pessoas portadoras de doenças infecto-contagiosas, tal interpretação da lei é materialmente inconstitucional por violação do art. 27.°, n.° 1, da C.R.P..
14 — Definindo a Lei n.° 27/2006, de 03-07 (Lei de Bases da Protecção Civil) "Acidente grave" como um acontecimento inusitado com efeitos relativamente limitados no tempo e no espaço, susceptível de atingir as pessoas e outros seres vivos, os bens ou o ambiente, mas estabelecendo no art. 5.°, n.° 1, al. a), o princípio da prioridade do interesse público relativo à protecção civil relativamente aos interesses da defesa nacional, segurança interna e saúde pública, podemos concluir que as situações graves de saúde pública, como a actual pandemia, não estão incluídas no interesse público relativo à protecção civil, logo, não estão incluídas nos conceitos de "acidente grave" e "catástrofe" a que se refere o art. 3.° da Lei de Protecção Civil.
15 — Daqui se pode também concluir que as Resoluções do Conselho de Ministros — e as Resoluções do Conselho do Governo Regional — que se fundaram na Lei de Bases da Protecção Civil para declarar "a situação de contingência e alerta, no âmbito da pandemia da doença COVID-19", nomeadamente as Resoluções do Conselho de Ministro n.°s 55-A/2020, de 31-07, 63-A/2020, de 14-08, 68-A/2020, de 28-08, e 70-A/2020, de 11-09 — revogadas pela Resolução do Conselho de Ministros n.° 88-A/2020, de 14-10, actualmente em vigor —, que prevêem no ponto 2 o «confinamento obrigatório, em estabelecimento de saúde, no respectivo domicilio ou noutro local definido pelas autoridades de saúde: (...) «a) Os doentes com COVID-19 e os infectados com SARS-CoV-2; (...) «b) Os cidadãos relativamente a quem a autoridade de saúde ou outros profissionais de saúde tenham determinado a vigilância activa», não têm fundamento legal, porquanto ***a Lei de Protecção Civil não se aplica a situações de perigo para a saúde pública.***
16 — Podemos, assim, concluir que as Resoluções do Conselho de Ministro n.°s 55-A/2020, de 31-07, 63-A/2020, de 14-08, 68-A/2020, de 28-08, 81/2020, de 29-09 — revogada esta última pela Resolução do Conselho de Ministros n.° 88-A/2020, de 14-10, actualmente em vigor —, e respectivo Anexo, que foram emitidas pelo Governo, no uso de competências administrativas, criaram um regime que restringe a liberdade dos cidadãos portadores de doenças infecto-contagiosas (quarentenas, isolamentos profilácticos, etc.) e, para reforçar a aplicação de uma privação da liberdade não permitida pela Constituição nem prevista em lei habilitante para as situações de portadores de doença contagiosa ou de perigo para a saúde pública, estabeleceram a cominação da prática de um crime de desobediência para tais violações e o agravamento da pena prevista para tal crime, violam, de forma directa, o art. 27.°, n.° 1, da C.R.P., pelo que, por inconstitucionais, deverão ser desaplicadas no caso concreto, ao contrário do pedido pela recorrente,
17 — Mantendo-se a decisão sub judice.
4. A recorrente é a autoridade regional de saúde, representada pela Direcção Regional da Saúde da Região Autónoma dos Açores.
Determina o Decreto-Lei n.º 11/93, de 1993-01-15, na sua versão actual (Estatuto do Serviço Nacional de Saúde) que (sublinhados nossos):
Artigo 1.º

O Serviço Nacional de Saúde, adiante designados por SNS, **é um conjunto ordenado e hierarquizado de instituições e de serviços oficiais prestadores de cuidados de saúde, funcionando sob a superintendência ou a tutela do Ministro da Saúde**.
Artigo 3.º
1 - O SNS organiza-se em regiões de saúde.
2 - As regiões de saúde dividem-se em sub-regiões de saúde, integradas por áreas de saúde.
Artigo 6.º
1 - **Em cada região de saúde há uma administração regional de saúde, adiante designada por ARS**.
2 - As ARS têm personalidade jurídica, autonomia administrativa e financeira e património próprio.
3 - As ARS têm funções de planeamento, distribuição de recursos, orientação e coordenação de actividades, gestão de recursos humanos, apoio técnico e administrativo e ainda de avaliação do funcionamento das instituições e serviços prestadores de cuidados de saúde.
4 – (…).
Por seu turno, estipula o Decreto-Lei n.º 22/2012
Artigo 1.º
1 - As Administrações Regionais de Saúde, I. P., abreviadamente designadas por **ARS**, I.P**., são institutos públicos integrados na administração indirecta do Estado**, dotados de autonomia administrativa, financeira e património próprio.
2 - **As ARS, I. P., prosseguem as suas atribuições, sob superintendência e tutela do membro do Governo responsável pela área da saúde.**
3 - As ARS, I.P., regem-se pelas normas constantes do presente decreto-lei, pelo disposto na lei-quadro dos institutos públicos e no Estatuto do Serviço Nacional de Saúde e pelas demais normas que lhe sejam aplicáveis.
Artigo 3.º
1 - As ARS, I. P., têm por missão garantir à população da respectiva área geográfica de intervenção o acesso à prestação de cuidados de saúde, adequando os recursos disponíveis às necessidades e cumprir e fazer cumprir políticas e programas de saúde na sua área de intervenção.
2 - São atribuições de cada ARS, I. P., no âmbito das circunscrições territoriais respectivas:
a) Executar a política nacional de saúde, de acordo com as políticas globais e sectoriais, visando o seu ordenamento racional e a optimização dos recursos;
b) Participar na definição das medidas de coordenação intersectorial de planeamento, tendo como objectivo a melhoria da prestação de cuidados de saúde;
c) Colaborar na elaboração do Plano Nacional de Saúde e acompanhar a respectiva execução a nível regional;
d) Desenvolver e fomentar actividades no âmbito da saúde pública, de modo a garantir a protecção e promoção da saúde das populações;
e) Assegurar a execução dos programas de intervenção local com vista à redução do consumo de substâncias psicoactivas, a prevenção dos comportamentos aditivos e a diminuição das dependências;
f) Desenvolver, consolidar e participar na gestão da Rede Nacional de Cuidados

Continuados Integrados de acordo com as orientações definidas;
g) Assegurar o planeamento regional dos recursos humanos, financeiros e materiais, incluindo a execução dos necessários projectos de investimento, das instituições e serviços prestadores de cuidados de saúde, supervisionando a sua afectação;
h) Elaborar, em consonância com as orientações definidas a nível nacional, a carta de instalações e equipamentos;
i) Afectar, de acordo com as orientações definidas pela Administração Central do Sistema de Saúde, I. P., recursos financeiros às instituições e serviços prestadores de cuidados de saúde integrados ou financiados pelo Serviço Nacional de Saúde e a entidades de natureza privada com ou sem fins lucrativos, que prestem cuidados de saúde ou actuem no âmbito das áreas referidas nas alíneas e) e f);
j) Celebrar, acompanhar e proceder à revisão de contratos no âmbito das parcerias público-privadas, de acordo com as orientações definidas pela Administração Central do Sistema de Saúde, I. P., e afectar os respectivos recursos financeiros;
l) Negociar, celebrar e acompanhar, de acordo com as orientações definidas a nível nacional, os contratos, protocolos e convenções de âmbito regional, bem como efectuar a respectiva avaliação e revisão, no âmbito da prestação de cuidados de saúde bem como nas áreas referidas nas alíneas e) e f);
m) Orientar, prestar apoio técnico e avaliar o desempenho das instituições e serviços prestadores de cuidados de saúde, de acordo com as políticas definidas e com as orientações e normativos emitidos pelos serviços e organismos centrais competentes nos diversos domínios de intervenção;
n) Assegurar a adequada articulação entre os serviços prestadores de cuidados de saúde de modo a garantir o cumprimento da rede de referenciação;
o) Afectar recursos financeiros, mediante a celebração, acompanhamento e revisão de contratos no âmbito dos cuidados continuados integrados;
p) Elaborar programas funcionais de estabelecimentos de saúde;
q) Licenciar as unidades privadas prestadoras de cuidados de saúde e as unidades da área das dependências e comportamentos aditivos do sector social e privado;
r) Emitir pareceres sobre planos directores de unidades de saúde, bem como sobre a criação, modificação e fusão de serviços;
s) Emitir pareceres sobre a aquisição e expropriação de terrenos e edifícios para a instalação de serviços de saúde, bem como sobre projectos das instalações de prestadores de cuidados de saúde.
3 - Para a prossecução das suas atribuições, as ARS, I. P., podem colaborar entre si e com outras entidades do sector público ou privado, com ou sem fins lucrativos, nos termos da legislação em vigor.
5. **A providência de *habeas corpus* requerida insere-se no disposto no artº 220 do C.P.Penal, que tem a seguinte redacção:**
Habeas corpus em virtude de detenção ilegal
1 - Os detidos à ordem de qualquer autoridade podem requerer ao juiz de instrução da área onde se encontrarem que ordene a sua imediata apresentação judicial, com algum dos seguintes fundamentos:
a) Estar excedido o prazo para entrega ao poder judicial;
b) Manter-se a detenção fora dos locais legalmente permitidos;
c) Ter sido a detenção efectuada ou ordenada por entidade incompetente;

d) Ser a detenção motivada por facto pelo qual a lei a não permite.
2 - O requerimento pode ser subscrito pelo detido ou por qualquer cidadão no gozo dos seus direitos políticos.
3 - É punível com a pena prevista no artigo 382.º do Código Penal qualquer autoridade que levantar obstáculo ilegítimo à apresentação do requerimento referido nos números anteriores ou à sua remessa ao juiz competente.
6. Apreciando.
Estipula o artº 401 do C.P. Penal o seguinte:
1 - Têm legitimidade para recorrer:
a) O Ministério Público, de quaisquer decisões, ainda que no exclusivo interesse do arguido;
b) O arguido e o assistente, de decisões contra eles proferidas;
c) As partes civis, da parte das decisões contra cada uma proferidas;
d) Aqueles que tiverem sido condenados ao pagamento de quaisquer importâncias, nos termos deste Código, ou tiverem a defender um direito afectado pela decisão.
2 - Não pode recorrer quem não tiver interesse em agir.
7. A primeira questão que aqui se coloca é a da legitimidade da recorrente, em sede de recurso em processo-crime.
i. Estamos no âmbito de uma jurisdição criminal, cujo propósito é o de assegurar o efectivo exercício do *jus puniendi* do Estado, isto é, que se dedica à averiguação e decisão relativamente a comportamentos que constituam crime ou contra-ordenação.
É, nesse âmbito e atenta tal finalidade, que a Lei determina quem tem legitimidade para poder discutir a bondade de uma decisão proferida por um tribunal criminal.
ii. No caso, constatamos que a recorrente não é arguida, não é assistente e não formulou qualquer pedido de natureza cível que, face ao princípio da adesão, lhe determinasse a posição de demandante ou demandada.
iii. Assim, perante a Lei e atendendo ao rol de intervenientes que o legislador entendeu poderem ter legitimidade para intervir num processo neste tipo de jurisdição, em sede de recurso, teremos desde logo de concluir que carece a recorrente de legitimidade para poder vir discutir o teor de uma decisão judicial, neste contexto.
iv. Efectivamente, não se discute aqui a prática de qualquer crime, nem de qualquer ilícito de natureza contra-ordenacional, sendo certo que a questão das eventuais consequências a nível criminal, do reconhecimento de existência de uma detenção ilegal, é matéria que terá de ser discutida em sede própria – isto é, em inquérito que venha a ser aberto para tal fim, sendo completamente estranha à decisão da presente causa.
v. Concluímos, assim, que carece a recorrente de legitimidade para interpor recurso da decisão proferida pelo tribunal "a quo".
8. Independentemente da questão da legitimidade, constata-se que, de igual modo, carece a recorrente de interesse em agir.
i. Como decorre da jurisprudência e doutrina pacíficas a este respeito, o interesse em agir significa a necessidade de alguém ter de usar o mecanismo de recurso como modo de reacção **contra uma decisão que lhe acarrete uma desvantagem para os interesses que defende ou que tenha frustrado uma sua legítima expectativa ou benefício.**
ii. Ora, no caso presente, a pergunta é – a decisão proferida acarretou qualquer desvantagem para os interesses que a ARS defende? Ou uma sua legítima expectativa ou benefício?

A resposta é manifestamente negativa.
Senão, vejamos.
iii. A ARS **prossegue as suas atribuições, sob superintendência e tutela do membro do Governo responsável pela área da saúde**.
Assim, e desde logo, quer face às funções que lhe são cometidas, quer perante a manifesta hierarquização das mesmas, perante a tutela, terá de se concluir que nenhuma ARS prossegue um interesse próprio e autónomo, que lhe cumpra defender. Quem o prosseguirá, eventualmente, será o Ministro respectivo ou o Governo em que se insere, pois os "interesses" da ARS não serão seus, mas englobar-se-ão na política de saúde do ministério que tutela tal entidade.

Note-se, aliás que na definição das suas atribuições[1], não lhe é determinada qualquer específica função de defesa, de modo autónomo e em nome próprio, em sede judicial, de quaisquer interesses que se insiram nas suas funções que, no que respeita a actividades do foro criminal ou contra-ordenacional, são nenhumas...
iv. Por seu turno, o interesse que a própria recorrente pretende defender e que consta em sede de pedido, no final deste recurso – a validação do confinamento obrigatório dos requerentes, por serem portadores do vírus SARS -CoV-2 (AH___) e por estarem em vigilância activa, por exposição de alto risco, decretada pelas autoridades de saúde (SH__SWH__ e NK___) – é algo em si mesmo contraditório e que ultrapassa a finalidade e o âmbito de competências de um tribunal criminal.
Contraditório porque a recorrente não admite que confinamento corresponda a privação da liberdade. Se assim é, não se vislumbra em que sede funda a recorrente a competência de um tribunal criminal para validar "confinamentos". E fora do âmbito de actuação de um tribunal criminal, porque a este não compete proceder a decisões declarativas de validação de infecções ou doenças…
v. Finalmente, não se enxerga que legítima expectativa ou benefício tenha uma entidade sob a tutela de um órgão do Governo, visto frustrada, pela decisão ora posta em crítica.
vi. Do dito decorre que não tem a recorrente interesse em agir, razão pela qual, ao abrigo do disposto no nº2 do artº 401 do C.P. Penal, não pode recorrer da decisão proferida.
9. A decisão proferida pelo tribunal "a quo" de recebimento do presente recurso não vincula este tribunal (artº 414 do C.P.Penal), pelo que nada obsta a que se determine a sua rejeição.
10. Não obstante, e para paz e sossego das consciências, aditar-se-á ainda o seguinte:
Ainda que assim se não entendesse, **o recurso apresentado mostrar-se-ia manifestamente improcedente, pelas seguintes sucintas razões**:
i. Desde logo, pela exaustiva e acertada fundamentação exposta na decisão, pelo tribunal "a quo", cujo teor se subscreve na íntegra.
Na verdade, **face à Constituição e à Lei, não têm as autoridades de saúde poder ou legitimidade para privarem qualquer pessoa da sua liberdade - ainda que sob o rótulo de "confinamento", que corresponde efectivamente a uma detenção – uma vez que tal decisão só pode ser determinada ou validada por autoridade judicial, isto é, a competência exclusiva, face à Lei que ainda nos rege, para ordenar ou validar tal privação da liberdade, é acometida em exclusivo a um poder autónomo, à Magistratura Judicial.**
Daí decorre que, qualquer pessoa ou entidade que profira uma ordem, cujo

conteúdo se reconduza à privação da liberdade física, ambulatória, de outrem (qualquer que seja a nomenclatura que esta ordem assuma: confinamento, isolamento, quarentena, resguardo profiláctico, etc)**, que se não enquadre nas previsões legais, designadamente no disposto no artº 27 da CRP e sem que lhe tenha sido conferido tal poder decisório, por força de Lei - proveniente da AR, no âmbito estrito da declaração de estado de emergência ou de sítio, <u>respeitado que se mostre o princípio da proporcionalidade</u> - que a mandate e especifique os termos e condições de tal privação, estará a proceder a uma detenção ilegal, porque ordenada por entidade incompetente e porque motivada por facto pelo qual a lei a não permite (**diga-se, aliás, que esta questão já foi sendo debatida, ao longo dos tempos, a propósito de outros fenómenos de saúde pública, nomeadamente no que se refere à infecção por HIV e por tuberculose, por exemplo. E, que se saiba, nunca ninguém foi privado da sua liberdade, por suspeita ou certeza de padecer de tais doenças, precisamente porque a Lei o não permite).

É neste âmbito que se insere, sem qualquer sombra de dúvida, a situação em apreciação neste processo, sendo certo que o meio de defesa adequado, contra detenções ilegais, se subsume ao recurso a pedido de *habeas corpus*, previsto no artº 220, als. c) e d), do C.P.Penal.

E, correctamente, o tribunal "a quo" determinou a imediata libertação de quatro pessoas que se mostravam ilegalmente privadas de liberdade.

ii. Em segundo lugar, porque **o próprio pedido formulado no recurso, se mostra de impossível procedência**.

Senão, vejamos:

11. De facto, é pedido que seja validado "o confinamento obrigatório dos requerentes, **por serem portadores do vírus SARS-CoV-2** (AH___) e por estarem em vigilância activa, **por exposição de alto risco**, decretada pelas autoridades de saúde (SH__SWH__ e NK_)."

12. É com enorme espanto que este tribunal se confronta com tal pedido, especialmente se tivermos em atenção que a recorrente exerce a sua actividade no sector da saúde. **Desde quando é que compete a um tribunal fazer diagnósticos clínicos, por sua própria iniciativa e com base nos eventuais resultados de um teste? Ou à ARS? Desde quando é que o diagnóstico de uma doença é feito por decreto ou por lei?**

13. Como a recorrente tem mais do que obrigação de saber, **um diagnóstico é um acto médico, da exclusiva responsabilidade de um médico**.

É isso que resulta inequívoca e peremptoriamente do Regulamento n.º 698/2019, de 5.9 (regulamento que define os actos próprios dos médicos), publicado em DR.

Aí se determina, de modo imperativo (o que impõe o seu acatamento por todos, incluindo a recorrente) que (sublinhados nossos):

Artigo 1.º

Objecto

O presente regulamento define os actos profissionais próprios dos médicos, a sua responsabilidade, autonomia e limites, no âmbito do respectivo desempenho.

Artigo 3.º

Habilitação

1 — **<u>O médico é o profissional legalmente habilitado ao exercício da medicina</u>**, **<u>capacitado para o diagnóstico</u>**, tratamento, prevenção ou recuperação **de doenças e**

outros problemas de saúde, e apto a prestar cuidados e a intervir sobre indivíduos, conjuntos de indivíduos ou grupos populacionais, doentes ou saudáveis, tendo em vista a protecção, melhoria ou manutenção do seu estado e nível de saúde.
2 — **Os médicos possuidores de inscrição em vigor na Ordem dos Médicos são os únicos profissionais que podem praticar os actos próprios dos médicos, nos termos do Estatuto da Ordem dos Médicos,** aprovado pelo Decreto -Lei n.º 282/77, de 5 de Julho, com as alterações que lhe foram introduzidas pela Lei n.º 117/2015, de 31 de agosto e do presente regulamento.
Artigo 6.º
Acto médico em geral
1 — **O acto médico consiste na actividade diagnóstica**, **prognóstica**, **de vigilância**, de investigação, de perícias médico-legais, de codificação clínica, de auditoria clínica, **de prescrição e execução** de medidas terapêuticas farmacológicas e não farmacológicas, **de técnicas médicas**, cirúrgicas e de reabilitação, de promoção da saúde e prevenção da doença em todas as suas dimensões, designadamente física, mental e social das pessoas, grupos populacionais ou comunidades, no respeito pelos valores deontológicos da profissão médica.
Artigo 7.º
Acto de diagnóstico
A identificação de uma perturbação, doença ou do estado de uma doença pelo estudo dos seus sintomas e sinais e análise dos exames efectuados constitui um procedimento base em saúde que deve ser realizado por médico e, em cada área específica, por médico especialista e visa a instituição da melhor terapêutica preventiva, cirúrgica, farmacológica, não farmacológica ou de reabilitação.
14. Mesmo ao abrigo da Lei de Saúde Mental, Lei n.º 36/98, de 24 de Julho, o diagnóstico da patologia que pode levar ao internamento compulsivo, é obrigatoriamente realizado por médicos especialistas e o seu juízo técnico-científico - inerente à avaliação clínico-psiquiátrica - está subtraído à livre apreciação do juiz (vide artºs 13 nº3, 16 e 17 da dita Lei).
15. Assim, qualquer diagnóstico ou qualquer acto de vigilância sanitária (como é o caso da determinação de existência de infecção viral e de alto risco de exposição, que se mostram abrangidas nestes conceitos) feitos sem observação médica prévia aos requerentes, sem intervenção de médico inscrito na OM (que procedesse à avaliação dos seus sinais e sintomas, bem como dos exames que entendesse adequados à sua condição), viola tal Regulamento, assim como o disposto no artº 97 do Estatuto da Ordem dos Médicos, sendo passível de configurar o crime p. e p. pelo artº 358 al.b) (Usurpação de funções) do C.Penal, se ditado por alguém que não tem tal qualidade, isto é, que não é médico inscrito na Ordem dos Médicos.
Viola igualmente o nº1 do artº 6º da Declaração Universal sobre Bioética e Direitos Humanos, que Portugal subscreveu e se mostra interna e externamente obrigado a respeitar, uma vez que se não mostra junto aos autos nenhum documento comprovativo de ter sido prestado o consentimento esclarecido que essa Declaração impõe.
Mostra-se assim claro que a prescrição de métodos auxiliares de diagnóstico (como é o caso dos testes de detecção de infecção viral), bem como o diagnóstico quanto à existência de uma doença, relativamente a toda e qualquer pessoa, é matéria que não pode ser realizada por Lei, Resolução, Decreto, Regulamento ou qualquer outra via

normativa, por se tratarem de actos que o nosso ordenamento jurídico reserva à competência exclusiva de um médico, sendo certo que este, no aconselhamento do seu doente, deverá sempre tentar obter o seu consentimento esclarecido.
16. No caso que ora nos ocupa, não há qualquer indicação nem prova, de tal diagnóstico ter sido efectivamente realizado por profissional habilitado nos termos da Lei e que tivesse actuado de acordo com as boas práticas médicas.
Efectivamente, o que decorre dos factos dados como assentes, é que nenhum dos requerentes foi sequer visto por um médico, o que se mostra francamente inexplicável, face à invocada gravidade da infecção.
17. Na verdade, o único elemento que consta nos factos provados, a este respeito, é a realização de testes RT-PCR, sendo que um deles apresentou um resultado positivo em relação a uma das requerentes.
i. Ora, face à actual evidência científica, esse teste mostra-se, só por si, incapaz de determinar, sem margem de dúvida razoável, que tal positividade corresponde, de facto, à infecção de uma pessoa pelo vírus SARS-CoV-2, por várias razões, das quais destacamos duas (a que acresce a questão do *gold standard* que, pela sua especificidade, nem sequer abordaremos):
Por essa fiabilidade depender do número de ciclos que compõem o teste;
Por essa fiabilidade depender da quantidade de carga viral presente.
ii. Efectivamente, os testes RT-PCR (Reacção em cadeia da polimerase), testes de biologia molecular que detectam o RNA do vírus, comumente utilizados em Portugal para testar e enumerar o número de infectados (após recolha nasofaríngea), são realizados por amplificação de amostras, através de ciclos repetitivos.
Do número de ciclos de tal amplificação, resulta a maior ou menor fiabilidade de tais testes.
iii. E o problema é que essa fiabilidade se mostra, em termos de evidência científica (e neste campo, o julgador terá de se socorrer do saber dos peritos na matéria) mais do que discutível.
É o que resulta, entre outros, do muito recente e abrangente estudo *Correlation between 3790 qPCR positives samples and positive cell cultures including 1941 SARS-CoV-2 isolates*, by Rita Jaafar, Sarah Aherfi, Nathalie Wurtz, Clio Grimaldier, Van Thuan Hoang, Philippe Colson, Didier Raoult, Bernard La Scola, Clinical Infectious Diseases, ciaa1491,https://doi.org/10.1093/cid/ciaa1491,em https://academic.oup.com/cid/advance-article/doi/10.1093/cid/ciaa1491/5912603, publicado em finais de Setembro deste ano, pela *Oxford Academic*, realizado por um grupo que reúne alguns dos maiores especialistas europeus e mundiais na matéria.

Nesse estudo conclui-se[2], em tradução livre:
“A um limiar de ciclos (ct) de 25, cerca de 70% das amostras mantém-se positivas na cultura celular (i.e. estavam infectadas): num ct de 30, 20% das amostras mantinham-se positivas; num ct de 35, 3% das amostras mantinham-se positivas; e num ct acima de 35, nenhuma amostra se mantinha positiva (infecciosa) na cultura celular (ver diagrama).
Isto significa que se uma pessoa tem um teste PCR positivo a um limiar de ciclos de 35 ou superior (como acontece na maioria dos laboratórios do EUA e da Europa), as probabilidades de uma pessoa estar infectada é menor do que 3%. A probabilidade de a pessoa receber um falso positivo é de 97% ou superior”.

iv. O que decorre destes estudos é simples – **a eventual fiabilidade dos testes PCR realizados depende, desde logo, do limiar de ciclos de amplificação que os mesmos comportam,** de tal modo que, até ao limite de 25 ciclos, a fiabilidade do teste será de cerca de 70%; se forem realizados 30 ciclos, o grau de fiabilidade desce para 20%; se se alcançarem os 35 ciclos, o grau de fiabilidade será de 3%.
v. Ora, no caso presente, **ignora-se qual o número de ciclos de amplificação com que são realizados os testes PCR em Portugal, incluindo Açores e Madeira,** uma vez que não nos foi possível encontrar qualquer recomendação ou limite a esse respeito.
vi. Por seu turno, num estudo também muito recente de Elena Surkova, Vladyslav Nikolayevskyy e Francis Drobniewski, acessível em https://www.thelancet.com/journals/lanres/article/PIIS2213-2600(20)30453-7/fulltext, publicado no igualmente prestigiado *The Lancet, Respiratory Medicine*, refere-se (para além das múltiplas questões que a própria precisão do teste suscita, quanto à específica detecção do vírus sars-cov 2, por fortes dúvidas quanto ao cumprimento do chamado *gold standard*) que (tradução livre):
"Qualquer teste de diagnóstico **deve ser interpretado no contexto da possibilidade efectiva da doença, existente antes da sua realização**. Para Covid-19, essa decisão de realização do teste, depende da prévia avaliação da existência de sintomas, história médica anterior de Covid 19 ou presença de anticorpos, qualquer potencial exposição a essa doença e não verosimilhança de outro possível diagnóstico."[3]
"Uma das potenciais razões para a apresentação de resultados positivos poderá residir no prolongado derramamento de RNA viral, que se sabe poder estender-se por semanas, após a recuperação, naqueles que foram anteriormente expostos ao SARS-CoV-2. **Todavia, e mais relevantemente, não existem dados científicos que sugiram que baixos níveis de RNA viral por RT-PCR equivalham a infecção, excepto se a presença de partículas virais infecciosas tiver sido confirmada através de métodos de cultura laboratorial**. Em síntese, testes Covid-19 que acusem falsos positivos mostram-se cada vez mais prováveis, no actual panorama climático epidemiológico do Reino Unido, com consequências substanciais a nível pessoal, do sistema de saúde e societário."[4]
18. Assim, existindo tantas dúvidas científicas, expressas por peritos na matéria, que são as que aqui importam, quanto à fiabilidade de tais testes, ignorando-se os parâmetros da sua realização e não havendo nenhum diagnóstico realizado por um médico, no sentido da existência de infecção e de risco, nunca seria possível a este tribunal determinar que AH___ era portadora do vírus SARS-CoV-2, nem que SH__SWH__ e NK_ tivessem tido exposição de alto risco.
19. Em síntese final dir-se-á que, uma vez que o recurso interposto se mostra inadmissível, por falta de legitimidade e por falta de interesse em agir por parte da recorrente, bem como manifestamente improcedente, terá de ser rejeitado, ao abrigo do disposto nos artºs 401 nº1 al. a), 417 nº6 al. b) e artº420 nº1 als. a) e b), todos do C.P. Penal.

iv – decisão.

Face ao exposto, e ao abrigo do vertido nos artigos 417.º, n.º 6, al. b) e 420 nº1 als. a) e b), ambos do Código de Processo Penal, rejeita-se o recurso interposto por **AUTORIDADE REGIONAL DE SAÚDE,** representada pela Direcção Regional da Saúde da Região Autónoma dos Açores**.**

Nos termos do n.º 3 do artigo 420.º do C.P.Penal, condena-se a recorrente na sanção processual de 4 UC, bem como na T.J de 4 UC e nas custas.
Dê imediato conhecimento ao tribunal "a quo" do teor do presente acórdão.

Lisboa, **11 de Novembro de 2020**
Margarida Ramos de Almeida
Ana Paramés

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[1][1] 2 - São atribuições de cada ARS, I. P., no âmbito das circunscrições territoriais respectivas:
a) Executar a política nacional de saúde, de acordo com as políticas globais e sectoriais, visando o seu ordenamento racional e a optimização dos recursos;
b) Participar na definição das medidas de coordenação intersectorial de planeamento, tendo como objectivo a melhoria da prestação de cuidados de saúde;
c) Colaborar na elaboração do Plano Nacional de Saúde e acompanhar a respectiva execução a nível regional;
d) Desenvolver e fomentar actividades no âmbito da saúde pública, de modo a garantir a protecção e promoção da saúde das populações;
e) Assegurar a execução dos programas de intervenção local com vista à redução do consumo de substâncias psicoactivas, a prevenção dos comportamentos aditivos e a diminuição das dependências;
f) Desenvolver, consolidar e participar na gestão da Rede Nacional de Cuidados Continuados Integrados de acordo com as orientações definidas;
g) Assegurar o planeamento regional dos recursos humanos, financeiros e materiais, incluindo a execução dos necessários projectos de investimento, das instituições e serviços prestadores de cuidados de saúde, supervisionando a sua afectação;
h) Elaborar, em consonância com as orientações definidas a nível nacional, a carta de instalações e equipamentos;
i) Afectar, de acordo com as orientações definidas pela Administração Central do Sistema de Saúde, I. P., recursos financeiros às instituições e serviços prestadores de cuidados de saúde integrados ou financiados pelo Serviço Nacional de Saúde e a entidades de natureza privada com ou sem fins lucrativos, que prestem cuidados de saúde ou actuem no âmbito das áreas referidas nas alíneas e) e f);
j) Celebrar, acompanhar e proceder à revisão de contratos no âmbito das parcerias público-privadas, de acordo com as orientações definidas pela Administração Central do Sistema de Saúde, I. P., e afectar os respectivos recursos financeiros;
l) Negociar, celebrar e acompanhar, de acordo com as orientações definidas a nível nacional, os contratos, protocolos e convenções de âmbito regional, bem como efectuar a respectiva avaliação e revisão, no âmbito da prestação de cuidados de saúde bem como nas áreas referidas nas alíneas e) e f);
m) Orientar, prestar apoio técnico e avaliar o desempenho das instituições e serviços prestadores de cuidados de saúde, de acordo com as políticas definidas e com as orientações e normativos emitidos pelos serviços e organismos centrais competentes nos diversos domínios de intervenção;
n) Assegurar a adequada articulação entre os serviços prestadores de cuidados de saúde de modo a garantir o cumprimento da rede de referenciação;
o) Afectar recursos financeiros, mediante a celebração, acompanhamento e revisão de contratos no âmbito dos cuidados continuados integrados;
p) Elaborar programas funcionais de estabelecimentos de saúde;
q) Licenciar as unidades privadas prestadoras de cuidados de saúde e as unidades da área das dependências e comportamentos aditivos do sector social e privado;
r) Emitir pareceres sobre planos directores de unidades de saúde, bem como sobre a criação, modificação e fusão de serviços;
s) Emitir pareceres sobre a aquisição e expropriação de terrenos e edifícios para a instalação de serviços de saúde, bem como sobre projectos das instalações de prestadores de cuidados de saúde.

[2] *"that at a cycle threshold (ct) of 25, about 70% of samples remained positive in cell culture (i.e. were infectious); at a ct of 30, 20% of samples remained positive; at a ct of 35, 3% of samples remained positive; and at a ct above 35, no sample remained positive (infectious) in cell culture (see diagram)*
***This means that if a person gets a "positive" PCR test result at a cycle threshold of 35 or higher** (as applied in most US labs and many European labs), **the chance that the person is infectious is less than 3%. The chance that the person received a "false positive" result is 97% or higher**.*

[3] Any diagnostic test result should be interpreted in the context of the pretest probability of disease. For COVID-19, the pretest probability assessment includes symptoms, previous medical history of COVID-19 or presence of antibodies, any potential exposure to COVID-19, and likelihood of an alternative diagnosis.1 When low pretest probability exists,

positive results should be interpreted with caution and a second specimen tested for confirmation.

[4] Prolonged viral RNA shedding, which is known to last for weeks after recovery, can be a potential reason for positive swab tests in those previously exposed to SARS-CoV-2. However, importantly, no data suggests that detection of low levels of viral RNA by RT-PCR equates with infectivity unless infectious virus particles have been confirmed with laboratory culturebased methods.7

To summarise, false-positive COVID-19 swab test results might be increasingly likely in the current epidemiological climate in the UK, with substantial consequences at the personal, health system, and societal levels (panel).

CORRECTED PROOF

Bilaga 7

Correlation Between 3790 Quantitative Polymerase Chain Reaction–Positives Samples and Positive Cell Cultures, Including 1941 Severe Acute Respiratory Syndrome Coronavirus 2 Isolates FREE

Rita Jaafar, Sarah Aherfi, Nathalie Wurtz, Clio Grimaldier, Thuan Van Hoang, Philippe Colson, Didier Raoult, Bernard La Scola ✉

Clinical Infectious Diseases, ciaa1491, https://doi.org/10.1093/cid/ciaa1491

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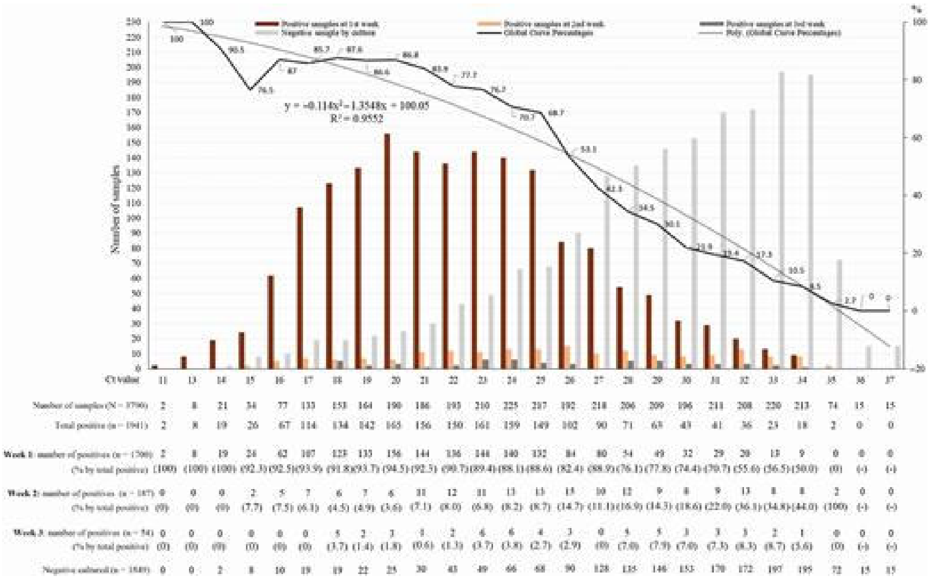
Issue Section: Correspondence

TO THE EDITOR—The outbreak of the coronavirus disease 2019 (COVID-19) pandemic due to severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) was declared a pandemic on 12 March 2020 by the World Health Organization [1]. A major issue related to the outbreak has been to correlate viral RNA load obtained after reverse-transcription polymerase chain reaction (RT-PCR) and expressed as the cycle threshold (Ct) with contagiousness and therefore duration of eviction from contacts and discharge from specialized infectious disease wards. Several recent publications, based on more than 100 studies, have attempted to propose a cutoff Ct value and duration of eviction, with a consensus at approximately Ct >30 and at least 10 days, respectively [2–5]. However, in an article published in *Clinical Infectious Diseases*, Bullard et al reported that patients could not be contagious with Ct >25 as the virus is not detected in culture above this value [6]. This limit was then evoked in the French media during an interview with a member of the French Scientific Council Covid-19 as a possible value above which patients are no longer contagious [7].

At the beginning of the outbreak, we correlated Ct values obtained using our PCR technique based on amplification of the E gene and the results of the culture [8]. Since the beginning of the pandemic, we have performed 250 566 SARS-CoV-2 RT-PCR for 179 151 patients, of whom 13 161 (7.3%) tested positive. Up to the end of May, 3790 of these samples, reported as positive on nasopharyngeal samples, were inoculated and managed for culture as previously described [8]. Of these 3790 inoculated samples, 1941 SARS-CoV-2 isolates could be obtained after the first inoculation or up to 2 blind subcultures. The correlation between the scanner values and the positivity of the culture allows us to observe that the image obtained with 10 times more isolates than in our preliminary work (1941 vs 129) does not change significantly (Figure 1). It can be observed that at

Ct = 25, up to 70% of patients remain positive in culture and that at Ct = 30 this value drops to 20%. At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive. Our Ct value of 35, initially based on the results obtained by RT-PCR on control negative samples in our laboratory and initial results of cultures [8], is validated by the results herein presented and is in correlation with what was proposed in Korea [9] and Taiwan [10]. We could observe that subcultures, especially the first one, allow an increasing percentage of viral isolation in samples with Ct values, confirming that these high Ct values are mostly correlated with low viral loads. From our cohort, we now need to try to understand and define the duration and frequency of live virus shedding in patients on a case-by-case basis in the rare cases when the PCR is positive beyond 10 days, often at a Ct >30. In any cases, these rare cases should not impact public health decisions.

Figure 1.



Percentage of positive viral cultures of severe acute respiratory syndrome coronavirus 2 polymerase chain reaction–positive nasopharyngeal samples from coronavirus disease 2019 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve. Abbreviations: Ct, cycle threshold; Poly., polynomial.

Notes

Ethical approval. The protocol was approved by the University Hospital Institute Méditerranée Infection Ethical Committee. All patients provided informed consent in accordance with the Declaration of Helsinki.

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Comments

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Bilaga 8

Brief Report | Open Access
Published: 27 April 2020

Viral RNA load as determined by cell culture as a management tool for discharge of SARS-CoV-2 patients from infectious disease wards

Bernard La Scola, Marion Le Bideau, Julien Andreani, Van Thuan Hoang, Clio Grimaldier, Philippe Colson, Philippe Gautret & Didier Raoult

European Journal of Clinical Microbiology & Infectious Diseases **39**, 1059–1061(2020)

Abstract

In a preliminary clinical study, we observed that the combination of hydroxychloroquine and azithromycin was effective against SARS-CoV-2 by shortening the duration of

viral load in Covid-19 patients. It is of paramount importance to define when a treated patient can be considered as no longer contagious. Correlation between successful isolation of virus in cell culture and Ct value of quantitative RT-PCR targeting E gene suggests that patients with Ct above 33–34 using our RT-PCR system are not contagious and thus can be discharged from hospital care or strict confinement for non-hospitalized patients.

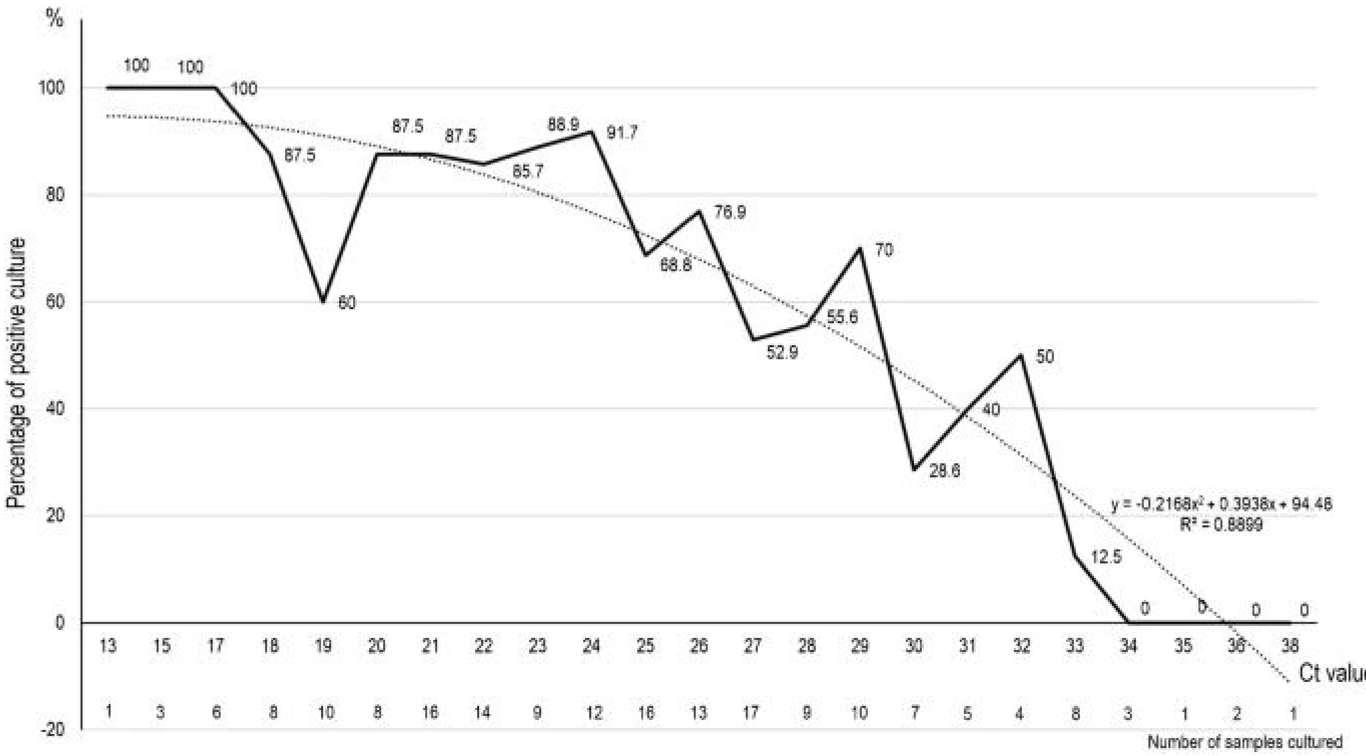
Introduction

An outbreak of an emerging disease (Covid-19) due to SARS-CoV-2 started in Wuhan, China, then rapidly spread in China, and was declared pandemic on March 12, 2020, by the WHO [1,2,3]. Currently, the overall case fatality rate is about 2.3% in China, which is likely an overestimate because most patients have mild symptoms and are thus not tested [4]. Because of a study showing that chloroquine and hydroxychloroquine inhibit SARS-CoV-2 in vitro, we tested hydroxychloroquine as a treatment in Covid-19 patients [5, 6]. Our results show that in treated patients, the nasopharyngeal viral load of SARS-CoV-2-infected patients was cleared in only 3 to 6 days. Our results also suggest a synergistic effect of the combination of hydroxychloroquine and azithromycin, two molecules previously demonstrated to be active in vitro against Zika and Ebola viruses [7,8,9,10] and to prevent severe respiratory tract infections when administered to patients suffering viral infection [11]. These results are of great importance because a recent paper has shown that the median duration of viral RNA detection in patients suffering from Covid-19 in China was 20 days, with the longest duration being 37 days [12]. We are now facing a massive influx of patients in need of treatment and hospitalization, ideally in infectious disease wards equipped with NSB3 modules with negative pressure. Thus, in addition to obtaining complete disappearance of virus RNA in respiratory samples, having a PCR-based indicator of loss of contagiousness is a major priority for discharge from infectious diseases ward. Based on a set of 183 samples from 155 patients, we observed a significant relationship between viral RNA load and culture positivity.

The Méditerranée Infection University Hospital Institute in Marseille is the reference center for highly infectious diseases for Southeastern France. It was the only center in this region with diagnostic tests available during the first weeks of the epidemic in France and received patients' samples from this whole area. From the day of the first positive test, on February 27 until March 12, 4384 clinical samples were tested by RT-PCR for 3466 patients. Since the beginning of this crisis and until now, on March 26, we inoculated 1049 samples and could obtain in culture 611 SARS-CoV-2 isolates. A total of 183 samples testing positive by RT-PCR, including 9 sputum samples and 174 nasopharyngeal swabs from 155 patients, were inoculated in cell cultures. SARS-CoV-2 RNA positivity in patient samples was assessed by real-time reverse transcription-PCR targeting the E gene, as previously described [13]. For all patients, 500 µL of nasopharyngeal swab fluid (Virocult, Elitech, France) or sputum sample were passed through 0.22-µm pore sized centrifugal filter (Merck Millipore, Darmstadt, Germany) and then were inoculated in 4 wells of 96-well culture microplates containing Vero E6 cells (ATCC CRL-1586) into Minimum Essential Medium culture medium with 4% fetal calf serum and 1% glutamine. All samples were inoculated between 4 and 10 h after sampling and kept at + 4 °C before processing. After centrifugation at 4000×*g*, microplates were incubated at 37 °C. They were observed daily for evidence of cytopathogenic effect. Two subcultures were performed weekly. Presumptive detection of virus in supernatant showing cytopathic effect was done using the SU5000 scanning electron microscope (Hitachi High-Tech Corporation, Tokyo, Japan) and then confirmed by specific RT-PCR targeting E gene. Variation of culture positivity rate was assessed statistically as the proportion of variance explained by Ct value and considered adequately fitted if the coefficient of determination (R^2 statistic) was > 50%.

Among the 183 samples inoculated in the studied period of time, 129 led to virus isolation. Of these, 124 samples had detectable cytopathic effect between 24 and 96 h. Blind subcultures allowed obtaining 5 additional isolates only. We observed a significant relationship between Ct value and culture positivity rate (Fig. 1). Samples with Ct values of 13–17 all led to positive culture. Culture positivity rate then decreased progressively according to Ct values to reach 12% at 33 Ct. No culture was obtained from samples with Ct ≥ 34. The 5 additional isolates obtained after blind subcultures had Ct between 27 and 34, thus consistent with low viable virus load.

Fig. 1



Percentage of positive viral culture of SARS-CoV-2 PCR-positive nasopharyngeal samples from Covid-19 patients, according to Ct value (plain line). The dashed curve indicates the polynomial regression curve

In the present work, we observe a strong correlation between Ct value and sample infectivity in a cell culture model. On the basis of this data, we can deduce that with our system, patients with Ct values equal or above 34 do not excrete infectious viral particles. It was observed that SARS-CoV-2 was detected up to 20 days after onset of symptoms by PCR in infected patients but that the virus could not be isolated after day 8 in spite of ongoing high viral loads of approximately 10^5 RNA copies/mL of sample, using the RT-PCR system used in the present study [14]. Progressive decrease of viral load over time is observed in all studies conducted in Covid-19 patients with positive detection being observed until 17–21 days after onset of symptoms, independently of symptoms [15]. These previous observations suggested that isolation of patients after diagnosis was mandatory. However, due to prolonged shedding of RNA in respiratory samples, the criteria for ending the isolation of a patient were not clear, and there was a need to correlate viral load to cultivable viruses. Our results show that in our system of RT-PCR, we can assess that patients with Ct equal or above 34 may be discharged. In 6 patients under the current therapeutic protocol used at our institute (hydroxychloroquine and azithromycin), Ct values $\geq$ 34 were obtained between days 2 and 4 post-treatment [6]. There is no previous correlation demonstrated between level viral load in respiratory samples and infectivity. However, this reduction is the basis of all procedures used for the validation of disinfectants [16]. One limitation of our work is that it cannot be extrapolated to other hospital centers since they use different systems of sample transport, of RNA extraction, and of PCR with different primers and probes; i.e. it has been suggested that sensitivity of amplification based on Gene E detection would be less sensitive than ORF1ab or N genes. We propose that each center perform its own correlation between culture results and viral RNA load from patients' samples. Another potential limitation is that nasopharyngeal swab fluid might be less representative than sputum samples. However, from the data obtained from patients rather tested in sputum, the viral load follows the same reduction with time of evolution than upper respiratory specimens [17].

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Ethics declarations

Conflict of interest

The authors declare that they have no conflict of interest.

Ethical approval

The protocol was approved by the ethical committee of the University Hospital Institute Méditerranée Infection (No. 2020-01).

Informed consent

All subjects gave a written informed consent in accordance with the Declaration of Helsinki.

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Keywords

- SARS-CoV2
- Covid-19
- RT-PCR
- Co-culture
- Viral load
- Correlation

Bilaga 9

CORMAN-DROSTEN REVIEW REPORT

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

Review report Corman-Drosten et al. Eurosurveillance 2020

November 27, 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.

Pieter 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.

The published RT-qPCR protocol for detection and diagnostics of 2019-nCoV and the manuscript suffer from numerous technical and scientific errors, including insufficient primer design, a problematic and insufficient RT-qPCR protocol, and the absence of an accurate test validation. Neither the presented test nor the manuscript itself fulfils the requirements for an acceptable scientific publication. Further, serious conflicts of interest of the authors are not

mentioned. Finally, the very short timescale between submission and acceptance of the publication (24 hours) signifies that a systematic peer review process was either not performed here, or of problematic poor quality. We provide compelling evidence of several scientific inadequacies, errors and flaws.

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

CONCISE REVIEW REPORT

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

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

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

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

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

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

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

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

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

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

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

1. The primers and probes:

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

2. The temperature at which all reactions take place:

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

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

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

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

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

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

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

MINOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

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

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

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

MAJOR CONCERNS WITH THE CORMAN-DROSTEN PAPER

A) BACKGROUND

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

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

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

B) METHODS AND RESULTS

1. Primer & Probe Design

1a) Erroneous primer concentrations

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

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

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

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

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

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

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

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

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

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

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

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

[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];

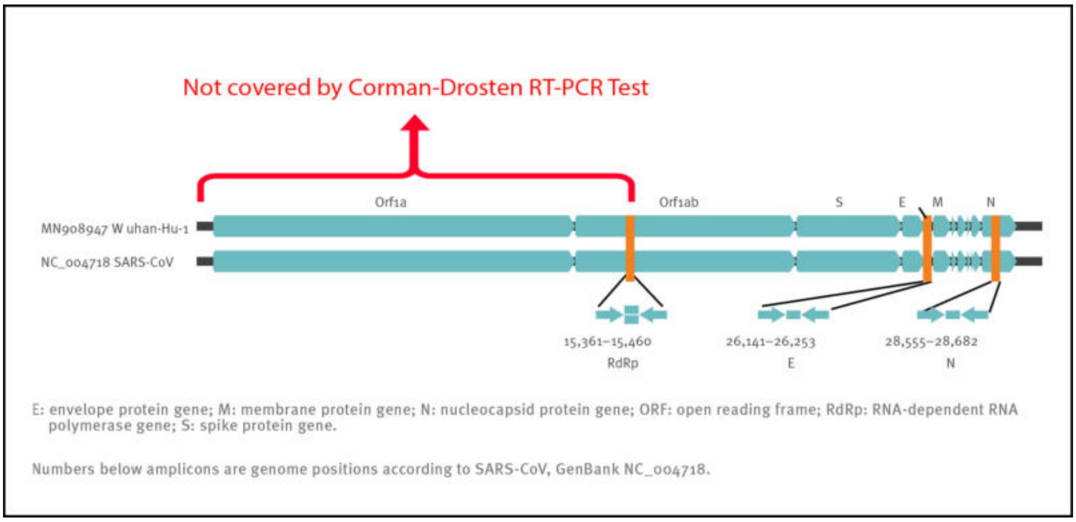
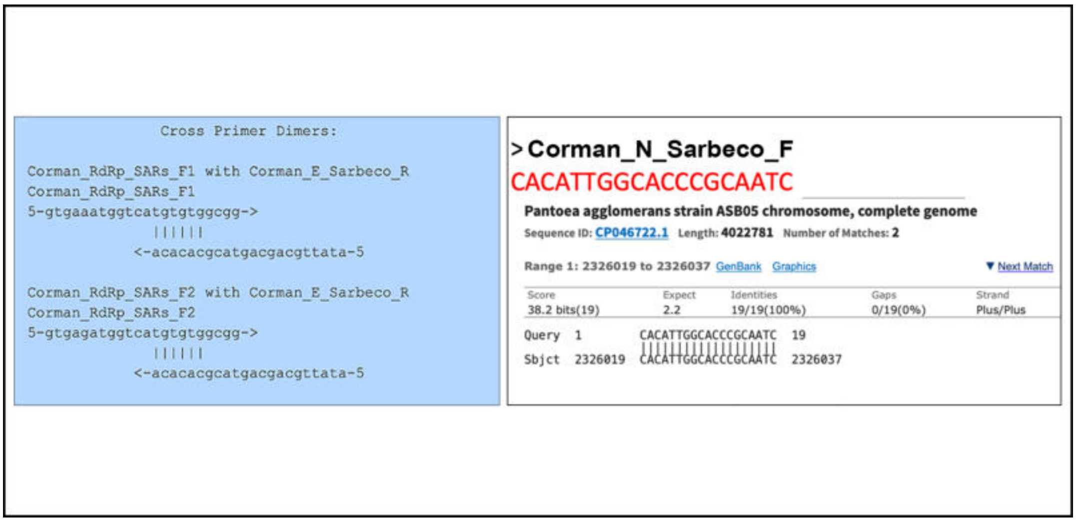


Figure 3: A test with Thermofischer's primer dimer web tool reveals that the RdRp forward primer has a 6bp 3`prime homology with Sarbeco E Reverse (left box). Another test reveals that there is a perfect match for one of the N-primers to a clinical pathogen (Pantoea) found in immuno-compromised patients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



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.

3

Primer pairs	Sequence (5´-3´)	GC Template strand	TM Length	Search in **MN908947** (first full genome from Wuhan, 12.01.2020) 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	

. The number of amplification cycles

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

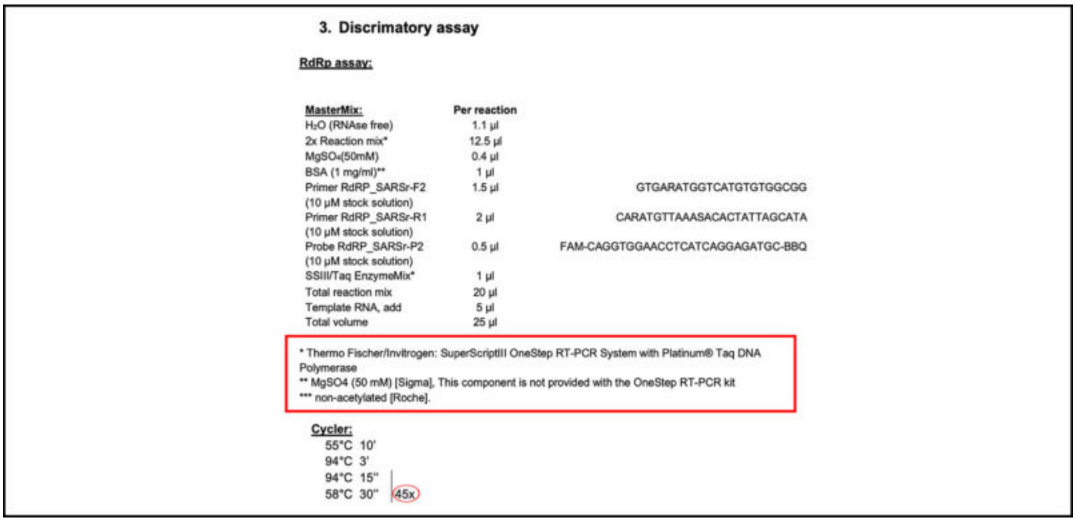
PCR data evaluated as positive after a Ct value of 35 cycles are completely unreliable.

Citing Jaafar et al. 2020 [3]: "At Ct = 35, the value we used to report a positive result for PCR, <3% of cultures are positive." **In other words, there was no successful virus isolation of SARS-CoV-2 at those high Ct values.**

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

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

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



3. Discrimatory assay

RdRp assay:

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

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

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

4. Biomolecular validations

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

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

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

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

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

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

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

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

Third, the Corman-Drosten paper states:

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

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

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

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

6. Standard Operational Procedure (SOP) is not available

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

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

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

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

> *“In four individual test reactions, weak initial reactivity was seen however they were negative upon retesting with the same assay. These signals were not associated with any particular virus, and for each virus with which initial positive reactivity occurred, there were other samples that contained the same virus at a higher concentration but did not test positive. Given the results from the extensive technical qualification described above, it was concluded that this initial reactivity was not due*

> *to chemical instability of real-time PCR probes and most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study." [1]*

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

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

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

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

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

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

9. Authors as the editors

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

SUMMARY CATALOGUE OF ERRORS FOUND IN THE PAPER

The Corman-Drosten paper contains the following specific errors:

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

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

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

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

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

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

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

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

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

10. We find severe conflicts of interest for at least four authors, in addition to the fact that two of the authors of the Corman-Drosten paper (Christian Drosten and Chantal Reusken) are members of the editorial board of Eurosurveillance. A conflict of interest was added on July 29 2020 (Olfert Landt is CEO of TIB-Molbiol; Marco Kaiser is senior researcher at GenExpress and serves as scientific advisor for TIB-Molbiol), that was not declared in the original version (and still is missing in the PubMed version); TIB-Molbiol is the company which was “the first” to produce PCR kits (Light Mix) based on the protocol published in the Corman-Drosten manuscript, and according to their own words, they distributed these PCR-test kits before the

publication was even submitted [20]; further, Victor Corman & Christian Drosten failed to mention their second affiliation: the commercial test laboratory "Labor Berlin". Both are responsible for the virus diagnostics there [21] and the company operates in the realm of real time PCR-testing.

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

CONCLUSION

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

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

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BRM: Planned and conducted the research, conceptualising the figures and manuscript.

MY: Proofreading the analyses and research.

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

KS: Conducted the analyses and research.

PMcS: Proofreading the analyses and research.

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Addendum

Update 2.12.2020:

Author Contribution Dr. Michael Yeadon changed to:
Proofreading the analyses and research.

Author Affiliation Kevin Mckernan changed to:
Medicinal Genomics.

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. **Madaload** says:
         December 1, 2020 at 8:35 pm

         Unless, of course, the government makes testing mandatory. Slovakia already had a few rounds of full scale (all citizens) AG testing. It wasn't mandatory per se. But if you didn't get tested, you didn't get a certificate and had to self isolate for 10 days. If you didn't get tested in the second round, same thing with 14 days isolation and your employer wouldn't let you work without the cert. After serious protests, the government now says AG positive results will be validated by PCR tests and 3 rounds of this "non mandatory" testing by Christmas.

         Reply

         1. **Lieveke** says:
            December 2, 2020 at 11:30 pm

            How sad ! Will be with Slovakia in mind…

            Reply

1. **Chantal** says:
December 3, 2020 at 5:46 pm

Hey is that u Liev?

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

1. **Liam King** says:
December 1, 2020 at 11:33 pm

Glad to hear you've been saying this all along.

Where were you shouting about this when you couldn't be heard?

Let me know so I can warn others to stay clear of such ineffectual places (and I will of course point them here instead).

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

2. **Evgeniy Gilenko** says:
December 1, 2020 at 5:39 pm

Think, the scope of the comment above is restricted to these two subtopics. Even though the practice of approval processes and publishing of scientific articles relies in my opinion mainly just on scientific integrity of reviewers, which I personally would like to trust, and the with regards to the massive impact of the study – direct or not – on the economies, societies etc., I simply would assume, that at the moment of submission and publication there were no evidence of pandemic outbreak of the SARS-COV-2 and therefore no self-evident need for deeper review of the study. Shortly saying, they probably didn't think the study would have any significant impact on anything. What I am asking myself instead, is why the first critical review appears only on 27th of November and was not conducted or initiated by the journal itself?

Reply

1. **Ralph Nimmann** says:
December 2, 2020 at 9:07 pm

VERY good question "why the first critical review appears only on 27th of November and was not conducted or initiated by the journal itself?"
I added a summary of this peer review on
https://healthtruth.info/weve-got-it-all-wrong-fighting-the-virus/#pcr

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

1. **Tatjana Z.** says:
December 3, 2020 at 6:13 pm

Danke für die Antwort! Dann hoffe ich sehr, dass ihr auch gehört werdet!

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

1. **Eric Vieira** says:
December 6, 2020 at 10:42 am

The dark agenda seems to be coordinated by the U.N. and WHO. Great Reset? Agenda 21? Agenda 2030? The virus outbreak in Wuhan was even negated by WHO for a while, allowing the virus to spread worldwide. That the more SAR-Cov2 virus-specific primers as negative/positive controls were not recommended tells a tale by itself.

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

1. **Vlrdngr** says:
December 1, 2020 at 12:11 pm

What if... He is talking about something else entirely, and the whole covid story was just a smokescreen or a cover up?

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

1. **Eric Vieira** says:

December 6, 2020 at 10:48 am

I fully support this. It would be essential to also follow the careers of the authors of this retraction request paper, to see if they become victims of repressive measures…

Reply

Bobby says:

December 6, 2020 at 11:28 am

I see, that was my fault. Deleted prior argument of mine. I oversaw the " " ".
Sorry for that.

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

1. **E.M. Oneal** says:
December 1, 2020 at 9:33 am

My profound apologies! I had in fact submitted the comment to the other thread on the "Retraction request letter", not this one, and you had already accepted it there. Thank you for your prompt response.

Reply

Bobby says:
December 1, 2020 at 9:46 am

No problem at all, it's not the best comments widget around, so it can get confusing, but it serves the cause.

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

1. **E.M. ONeal** says:
December 1, 2020 at 9:52 am

Some remarks by Prof. Ulrike Kämmerer in an interview by the "Corona Ausschuss" meade it clear to me that there is another source of false positives that are not covered by the statistical definition.

There are extraneous factors that introduce contamination during the lab test procedure "on the bench" (or before!). Possibilities are the operator sneezing or positive control mnaterial finding its way into the test, or cross- contamination from other samples.

That could account for the same sample testing positive on a re-test and would also be a false positive. Although one would expect that such errors are excluded in an accredited lab.

There are myriad other potential sources of extraneous false positives, starting with test kit production (contaminated swabs?), through the sample collection from the subject, lab procedures, data analysis and reporting.

Reply

Bobby says:
December 1, 2020 at 9:57 am

Dr. Michael Yeadon is very dedicated to this topic complex of "industrial complex / mass testing by non-experts". Thank you for your remarks.

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 natürlich nicht ein. Überhaupt ist Selbstreferenzierung oft ein schlechtes wissenschaftliches Gegen-Argument.

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

3. **Chrisje** says:
December 1, 2020 at 10:51 am

Even trolls are targeting scientists. What's new?

Reply

4. **roland brautigam** says:
December 1, 2020 at 12:05 pm

It's clowns like you. You have the arrogance to claim that Peter Borger and Kevin McKernan and Mike Yeates don't know how primer concentrations work without coming with a counter argument?

Reply

5. **John Weir** says:
December 2, 2020 at 9:11 am

Please do not try to initiate a slanging match.

No-one will bite.

Reply

6. **Hanno** says:
December 2, 2020 at 10:01 pm

I Love your reply Stephen 🙂 Quackery never died, snakeoil was never short, times don't change, it all comes back in cycles…

Reply

7. **ura soul** says:
December 3, 2020 at 3:16 pm

I am horrified to read this appalling interjection into a hotly contested topic which accuses people of being dangerously reckless with peoples lives without even inserting any verifiable claims or evidence.

Reply

8. **Thomas Ellenberg** says:
December 3, 2020 at 7:12 pm

I'm more horrified by ad hominem spammers who can't formulate a valid counter argument.

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

1. **Arnold Achen** says:
December 1, 2020 at 10:25 am

Was Sie verlangen wird von den Behörden nicht gefördert, weil sie mit den Konzerninteressen verflochten sind, die eine Fortführung der inszenierten Pandemie forcieren:

https://www.corona-schadensersatzklage.de/corona-status-quo-erste-pcr-klage-ist-anhaengig-gemacht-worden/

Und warum sollen die Labors an mehr QC interessiert sein wenn sie aktuell durch die reine Massentestung ein Vermögen verdienen?

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. **roland brautigam** says:
   December 1, 2020 at 12:15 pm

How about responses to quotes 7 to 9?

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

1. **Lothar Lammfromm** says:
December 3, 2020 at 10:59 am

Yes, you are not a physician and you don't know anything about Covid-19-Science.

This sentence is pure rubbish:

"most patients max their viral load on day 3 post symptom onset"

No, usually (but this can vary) it is the day before (!) system onset.

Reply

1. **Lothar Lammfromm** says:
December 3, 2020 at 2:06 pm

symptom onset

Reply

2. **ShowMeTheFacts** says:
December 6, 2020 at 1:12 pm

"...what purpose does the PCR test serve?"

1) To maximize the Casedemic
2) To use as junk science to justify totalitarian policy (businesses restrictions, community activities, sports, etc.)
3) To use as junk science to quarantine non-infectious teachers, students, staff, etc.
4)…
5)…
6)…

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

2. **Julian** says:
December 4, 2020 at 3:40 pm

Well, you don't have to worry about MERS.

Because dr. Drosten himself said in a 2014 interview that PCR isn't suitable for detecting MERS.
He admits the PCR technique is too sensitive, which leads to an explosion in case numbers and that the media tends to blow up these case numbers out of proportion.

https://www.wiwo.de/technologie/forschung/virologe-drosten-im-gespraech-2014-die-who-kann-nur-empfehlungen-aussprechen/9903228-2.html

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

47. **Danae** says:
December 1, 2020 at 10:12 am

God bless you all for this incredible work. It is time to cleanse our system from monetarism which is subverting science, medical reserach, press and politics. We cannot have independent, impartial science if it is "financed/bribed" by groups of have clear conflicts of interest. This paper is an important step to stopp the attack on civil liberties and stands between many people being injured by vaccination or even death or infertility. No vaccination is without risks, there are no studies ever being done on how different vaccinations interact with each other. Where there is risk there must be choice.
God bless you and protect you and my he help us all to stay for truth, love and peace.

Reply

1.  **Eric Vieira** says:
December 6, 2020 at 11:14 am

What worries me is that no studies have been done wrt ADE (antibody induced enhancement). This effect has been observed with related SARS and MERS viruses in animal models. There are other corona viruses out there which were up to now not so lethal, but the situation could get to be much worse if ADE in vaccinated people occurs.

Reply

48. **Michael Wiedom** says:
December 1, 2020 at 11:12 am

Herzlichen Dank für diese hervorragende Arbeit. Bin gespannt, wie es jetzt weitergehen wird.

Reply

49. **Petra von Kopp** says:
December 1, 2020 at 11:40 am

Danke… Danke…

Reply

50. **OrAnd** says:
December 1, 2020 at 11:56 am

Thanks for your work, I do have a comprehension question regarding 3.: How can there be false positives at all by this PCR-test? In the text (3) and reference [2][3] I can see only points stating why there can be false negatives (not false positives). Am I missing something here?

Reply

1. **Kevin McKernan** says:
December 2, 2020 at 6:12 pm

We present evidence of promiscuous primers. These can create both False Negatives and False Positives. There are additional sources of FPs as the assay fails to discern infectious from non-infectious people and when used on asymptomatic people, the term 'case' must be infectious. This is exacerbated by the selection of a reduced number of assays and assays positioned on the 3′ region of the genome. Wolfel et al describes qPCR amplicons that do a better job discerning infectious from non-infectious people. Drosten is an author on this paper. This would have been a good time to withdraw the assays failing to discern the difference between infectious cases and non-infections false cases.

Reply

51. **Ruud Brouwer** says:
December 1, 2020 at 12:09 pm

Will this report be peer reviewed? I really doubt it will make it through

Reply

52. **RHB** says:
December 1, 2020 at 12:29 pm

Well done to all involved. Meticulous scrutiny with massive implications. Not my area of detailed expertise but, if I'm reading broadly right, could even bring down the leadership of the country I'm posting from.

Substantiates gut feeling going right back to March, as per blog comments by "Ancient Briton," "RHB" and "Aweson Walles" on Derek Lowe's In the Pipeline blog (dominated March onwards by virus postings):

https://blogs.sciencemag.org/pipeline/archives/2020/03/12/real-information-a-public-good#comment-313468

16 March, 2020 at 9:09 am

https://blogs.sciencemag.org/pipeline/archives/2020/11/18/vaccine-possibilities

19 November, 2020 at 6:49 am

https://blogs.sciencemag.org/pipeline/archives/2020/11/23/oxford-az-vaccine-efficacy-data#comment-333601

29 November, 2020 at 10:21 am

Reply

53. **roland brautigam** says:
December 1, 2020 at 1:13 pm

Bobby have you seen the response from Marion Koopmans and Adam Meijer:
https://www.rd.nl/vandaag/binnenland/pcr-test-overleeft-stortvloed-aan-kritiek-1.1718351

She claims molecular validation was done.
She claims peer review was done
She claims they started working on the protocol from 12 January when China released the genetic code
She claims the guidelines for the PCR test were changed by RIVM on 25 September but that these should not change the outcome. I have a few statistics which prove the opposite.
Adam Meijer states that false positives could be 0,5-4%. 4% is catastrophic already.
Article is under "conspiracy theory" header…

Reply

Bobby says:
December 1, 2020 at 2:22 pm

Dr. Peter Borger will give an answer on this soon. I'll notify you.

Reply

54. **Joerg** says:
December 1, 2020 at 3:21 pm

Hallo,
Danke fuer die Recherche, das Corman-Drosten et al.Paper war halt ein Schnellschuss.

Mich wuerde interessieren, ab wann (im Zeitverlauf, zB ab September2020?) es bessere Tests (weniger Kreuzreaktionen, weniger Falsch-Positive, 3Gen-Prinzip, spezifischer, Beachtung von Ct-Werten) in den Test-Laboratorien gibt? Gibt's ein Labormediziner, der hier mit liest?

Ich kann mir nicht vorstellen, dass eine Vielzahl von Laboren immer noch die gewobelten RdRp-Primer verwendet? Oder?

LG Joerg

Reply

1. **M. Hoffmann** says:
December 1, 2020 at 4:08 pm

Bessere Tests für was? Für ein Virus das unter Umständen gar nicht existiert–laut Angaben des CDC gibt es kein messbares Isolat von sars-cov-2 weltweit– oder sich funkionell nicht von üblichen Coronaviren unterscheidet und auf jeden Fall denen gegenüber keine höhere Mortalität aufweist?

Was soll das klinische Korrelat zum "Test" sein? Welche therapeutischen Massnahmen sollen auf Grund von welchem Testergebnis eingeleitet werden und ergeben dies überhaupt einen Sinn, außer Unterdrückung?

Haben Sie in der Vergangenheit je einen Test für Grippe oder schwere Erkältung durchgemacht und was hat das gebracht?

Laut der Dokumentation zu Event 201 handelt es sich ohnehin um eine globale Simulation zur Bereicherung von "öffentlich-privaten Partnerschaften".

Es ist die Jagd nach einem Phantom, das benutzt wird, schwere psychologische und wirtschaftliche Schäden anzurichten.

Reply

1. **Joerg** says:
   December 2, 2020 at 3:20 pm

   Vielen Dank, M. Hoffmann, dann wissen Sie also nicht, ob aktuelle RT-PCR-Tests noch auf dem hier kritisierten Paper beruhen bzw welche Verbesserungen hinsichtlich Spezifitaet, Genauigkeit inzwischen unternommen wurden, bzw Eingang in aktuelle RT-PCR-Kits gefunden haben?!
   Sonst jemand?

   Sie antworten dagegen auf Fragen, die ich nicht gestellt habe. Gerne aber meine Meinung dazu:
   a) "Virus das unter Umstaenden nicht existiert" "und auf jeden Fall denen gegenüber keine höhere Mortalität aufweist"
   Belege dazu? Sind erhoehte Mortalitaeten in anderen Laendern als in D also alle gefaket? Wie erklaeren Sie erhoehte Mortalitaeten (zB auf http://www.euromomo.eu/graphs-and-maps) bei unseren Nachbarn?
   b) "klinische Korrelat zum "Test" sein?"
   Wie waere es mit dem Versuch, die Verbreitung etwas einzudaemmen, um Gesundheitssysteme/Intensivbetten nicht zu ueberlasten? Also keine klinische Konsequenz sondern eine epidemiologische?!
   c) "Haben Sie in der Vergangenheit je einen Test für Grippe oder schwere Erkältung durchgemacht"
   Nein, warum auch? Gibt's denn solche? Bin nicht in der Risikogruppe 😉
   d) "Laut der Dokumentation zu Event 201"
   Sie meinen, http://www.weforum.org/great-reset/? Da haette ich viel zu tun, alle dubiosen Traeume, Stilblueten und Ideen im Internet zu verfolgen 😉
   e) "Es ist die Jagd nach einem Phantom, das benutzt wird, schwere psychologische und wirtschaftliche Schäden anzurichten."
   Wissen Sie, dass manche Kommentare mehr ueber den Kommentator aussagen als ihnen Sinn und Zweck innewohnt?
   Meinen Sie wirklich, alles geschieht aus boesartiger Taktik?
   Meist reichen Angst, Unwissenheit, Inkompetenz, Unfaehigkeit aus, um fast alles "gut gemeinte" zu erklaeren … meine Erfahrung jedenfalls.

Sie kaempfen gegen Windmuehlen?!
LG Joerg

Reply

2. **Kane** says:
December 3, 2020 at 11:05 am

Ähmm.. Quelle [3] dieses Reports spricht eindeutig von Isolaten "1941 SARS-CoV-2 isolates could be obtained"
Also du stimmst diesem Report hier zu, aber dessen Quellen lehnst du ab?
Versteh ich das so richtig?

Reply

1. **Joerg** says:
December 3, 2020 at 4:05 pm

@Kane,
ich weiss nicht, was du meinst? Wolltest du unter einem anderen Kommentar kommentieren?

Quelle [3] finde ich interessant und verstehe sie genauso wie du? (also es wurden sehr wohl viele Covid19-Isolate untersucht sowohl per RT-PCR als auch in Subkultur).
Bei RT-PCR gibt's halt eine gewisse Gefahr von Falschpositiven, auch gibt es einen laborspezifischen Effekt (Labore mit mehr / weniger Expertise?) aber es ist trotzdem die beste aller Nachweismethoden fuer epidemiologische Studien (Schnelligkeit, Durchsatz) und so zu tun, als wuerde da "Irgendwas" rumamplifiziert ist m.E. weiter weg von der Realitaet als 0,5-2%? Fehler.
Alle machen Fehler, nix ist vollkommen in dieser Welt.
Trotzdem ist es ein sinnvolles und berechtigtes Ansinnen fuer mehr Sensitivitaet und Spezifitaet beim Corona-Testen einzustehen! Ein erster Schritt waere viell. bei jedem Test auch den Ct-Wert mit zu liefern? und Getestete mit Ct >30 und ohne Symptome sollten berechtigterweise maulig werden!
LG Joerg

Reply

1. **Joerg** says:
December 3, 2020 at 4:10 pm

Ah, hab jetzt die Kommentarstruktur gecheckt, du antwortest auf M. Hoffmann.
Sorry, Joerg

2. **Kane** says:
December 4, 2020 at 5:28 am

Genau 😉
Ging mir nur um dieses "laut Angaben des CDC gibt es kein messbares

Isolat von sars-cov-2 weltweit" von M. Hoffmann, was eine Aussage des CDC von Juli fehlinterpretiert.

2. **roland brautigam** says:
December 2, 2020 at 4:12 pm

Schnellschuss?? Glaube das dass nichgt ganz war ist: https://pubmed.ncbi.nlm.nih.gov/23231891/

Aus 2012!

Reply

55. **Francine** says:
December 1, 2020 at 5:06 pm

Ik heb een vraag over paragraaf 3 op blz 6. Waarom is daar letterlijk de persoonlijke mening over de PCR test overgenomen van de twee rechters van het Hof van beroep in Lissabon inzake het onrechtmatig in quarantaine plaatsen van vier Duitse toeristen. De zaak waardoor Wybren van Haga meende te moeten melden in de Kamer dat de rechter in Portugal de PCR test naar de prullenbak heeft verwezen.
Deze 'conclusie' is te lezen onder punt 17 van het arrest. Zij hebben deze aanname gemaakt na een foutief interpreteren van het onderzoek van Jafaar et al. Dit is dus geenszins de conclusie van de studie van Jafaar et al. Ook onder reviewed 3 maakt u een fout, het gaat hier niet primair om het aantal cycli (35) van de PCR waarboven besmetting < 3% is. Het gaat om het vermogen waarop besmette samples nog in staat zijn cellen in kweek te infecteren, dat was na 3 weken < 3% Dit om zicht te krijgen op duur van isolatie van covid-19 patiënten.
Morgen dient een zaak over de bemoeienis van beide rechters bij de Hoge Justitiële Raad van Portugal.
Volgens het SCM hebben de rechters hier hun bevoegdheden overschreden. Zij hadden geen opmerkingen moeten maken over de mate van betrouwbaarheid van de tests die momenteel worden gebruikt. Daarom zullen ze morgen op 2 december vragen moeten beantwoorden in een tuchtonderzoek door de Justitiële Raad, uitspraak door het Superior Council of Magistrature of de twee rechters juist gehandeld hebben.
Ik ben benieuwd of u hiervan op de hoogte bent en zal de uitspraak gevolgen voor de tekst op blz.6 paragraaf 3 hebben.

Reply

56. **Rich** says:
December 1, 2020 at 7:00 pm

These are theoretical weaknesses you may find in most scientific paper. As many of the reviewers are affiliated with biomedical institutions, why don't you just show experimentally the predicted consequences for false positives? When the assay is really that bad, this shouldn't be difficult!?

Reply

Bobby says:

December 1, 2020 at 7:58 pm

Sorry, this statement is completely irrelevant and refuted. Either you didn't read the review report or you forgot to pay attention.

Reply

1. **Rich** says:
December 1, 2020 at 9:43 pm

of course I did not spend too much time for this… but as it was already mentioned above a diagnostic test needs experimental validation, not whatever this is. Just show what you claim. It would be so easy to do this.

Reply

1. **Adam** says:
December 2, 2020 at 7:20 pm

"of course I did not spend too much time for this… "… so why did you leave a comment? Sometimes it is better to su. The authors did spend a lot of time to identirfy the weaknesses of this "study". It seems we have here a Drosten Fan Boy? For you this is almost religious…

Reply

1. **Rich** says:
December 3, 2020 at 8:36 am

Yes, they spend a lot of time to selectively search for minor weaknesses and of course they did not do any wet lab experiments because as apparently they have experience with qPCR, they know that in the end none of this will have a great effect, that the PCR works and that there are no "consequences for false positives" or whatsoever.

2. **Kevin McKernan** says:
December 4, 2020 at 7:07 am

See my comment on the thread.
We don't need to perform any experiments as we have provided 4 manuscripts that have already documented this effect.

Reply

Bobby says:
December 1, 2020 at 7:55 pm

What a weak comment. That's like asking why are you "running a website and making sure that nothing illegal is posted"? Failed troll-attempt.

Reply

57. **Francine** says:

December 1, 2020 at 9:07 pm

Ik zie dat mijn commentaar is geweigerd, kunt u mij zeggen wat de reden is van het weigeren?

Reply

 **Bobby** says:
December 1, 2020 at 9:16 pm

Sorry, oversaw your comment, have approved it now. We will come back to your point and notify you.

Reply

1. **Francine** says:
   December 1, 2020 at 9:37 pm

   Dankuwel

   Reply

58. **Francine** says:
    December 1, 2020 at 9:32 pm

    Sorry te snel gereageerd, ik wacht uw antwoord af

    Reply

59. **Carlyn Mildrum** says:
    December 2, 2020 at 2:29 am

    immaculate article

    Reply

60. **Bölecke** says:
    December 2, 2020 at 6:24 am

    Ich bin begeistert. Jetzt weiß ich warum die (unsere ?) Regierung nicht mit schlüssigen Erklärungen die Situation beschrieben hat.

    Reply

61. **Kim ten Napel** says:
    December 2, 2020 at 8:12 am

    Following this Corman/ Drosten retraction paper with interest and hope for a better world.
    How this ONE PCR test can cause such panic and be used to control the people is remarkable,as this one PCR test has so much power why hasn't it been tested and retested by every country.
    I applaud Peter Borger & Co for this admirable difficult investigation.

Reply

62. **John Weir** says:
December 2, 2020 at 9:04 am

Not a direct comment on the paper in question but the following is a statement by C. Whitty made in an interview with the BMJ.

"For the great majority of the infections I've dealt with—and I'm an
infectious disease epidemiologist—you never get herd immunity.
You don't get it for malaria, you don't get it for HIV, you don't get
it for Ebola."

With particular reference to HIV , am I the only person to see this statement as utterly ridiculous ?

Expert comment if someone would be so kind.

Sorry this is somewhat off-topic.

Reply

63. **Dr. med. Karla Lehmann** says:
December 2, 2020 at 9:21 am

Herzlichen Dank für Ihre äußerst wertvolle und notwendige kritische Einschätzung dieses CormanDrosten-Papers. Ihre Arbeit hat mir die Zuversicht zurückgegeben, daß es noch Wissenschaftler gibt, die die Dinge hinterfragen und der Sache auf den Grund gehen.

Reply

64. **John Weir** says:
December 2, 2020 at 10:09 am

Possible correction required.

"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). "

Should this be "Figure 2" instead of "Table 2" ?

Thanks

Reply

65. **Inhope** says:
December 2, 2020 at 10:38 am

Does any of you, the authors, SPEAK to humans you know?!
If you have any credibility around you, why haven't you disseminated all this personally?
You know, word of mouth is very potent! The interlocutors would diffuse it further with something like this:
"I know this person. I trust them. I will act as they advise." And so on.
I guess you are just tinkering with details.

Because if you knew that "viruses" are not real, you would have spoken long ago, and this report would have been unnecessary!
I am close to dying from old age. I have had time to acquire knowledge in more fields than anyone I heard of. Quantum Physics, Artificial Intelligence/Robotics, Electrodynamics, Philosophy of Science.
The last one mentioned provided the space where I, and others, could really take apart all the preposterous statements and outright lies of the "scientists" all over.
They all go to the brainwashing centers called universities and get poisoned by older brainwashed "professors". They have no chance of waking up later on because they never leave the fetid "educational" environment that raised them. One in a million does, but then they are afraid of losing their grants or professorships! So they continue in the complicity to murder and maim!
Have you heard of Thomas Kuhn?
Stefan Lanka?
One quit physics because of what I criticize, the other has been speaking for decades against his original domain: "virology".
Get informed.
And get honest.

Reply

1.  **roland brautigam** says:
   December 2, 2020 at 4:33 pm

   Understand your point well. However I believe that people like you (and me) who study or show a strong interest in QM especially on a level with neurology, quantum consciousness or nano-biology, our intellectual understanding is larger than 99,9999% of the general population. One in a million. It puts us in a position where sarcasm and anger in times like these are closer than enlightenment unfortunately. I have had doubts about the (people behind) the Drosten protocol from the beginning. There is just too much money involved. Hundreds of billions of dollars. Ab Oosterhaus for example was involved in the first studies in 2012. For me he is (one of) the Devil(s). I wish you lots of strength and especially happiness in your final years.

   Reply

66. **Peter Barnum** says:
December 2, 2020 at 11:44 am

I have two immediate questions:

1) The primer dimers you found are between one primer for the E gene assay and one for the RdRp assay. In real life testing, the two test are conducted in different wells, aren't they? So these two primers should never be in the same pot and therefore the potential dimers don't matter!?
2) Even if primer dimers form and are elongated by the polymerase, this would not lead to increased fluorescence, since a probe-based assay is used in contrast to intercalating dyes which would indeed just indicate any dsDNA being present.

Regards
Peter

Reply

Bobby says:
December 2, 2020 at 1:19 pm

We will be shortly in contact with you about your questions and remarks. Thank you very much.

Reply

1. **Peter Barnum** says:
December 2, 2020 at 8:45 pm

I am looking forward to that!

Reply

2. **Peter Barnum** says:
December 4, 2020 at 5:15 am

As long as you can answer other stuff and choose not to address my questions, I'm assuming that I am right and this is a blatant error in your report. So blatantly indeed that in combination with your ongoing unwillingness to discuss it, it makes me think that you knew this all along. This would mean that you knowingly pulished statements you knew were wrong, seriously questioning your scientific motivation!

Reply

Bobby says:
December 4, 2020 at 5:39 am

Kevin McKernan answered you. Your prior conclusions are irrelevant in this case.

Reply

1. **Peter Barnum** says:
December 4, 2020 at 5:47 am

I have so far not been able to find that answer. Was that under a twitter post and would you be so kind to share it with me? I am no longer ablo to see his own post, since he blocked me.

Bobby says:
December 4, 2020 at 5:49 am

https://cormandrostenreview.com/report/#comment-304

1. **Kevin McKernan** says:
December 4, 2020 at 4:29 am

Thank you for your comment.

We have evidence of labs reducing the number the amplicons in the test to cut costs from 3 amplicons to even a single amplicon used in Italy.

We are assuming a minority of people are multiplexing the test as well to cut costs but this is not material to our argument.

Even if you assume 100% singleplex utilization, there are multiple peer reviewed publications that demonstrate singleplex primer-dimers, false positives, and unreliable results. Are these dimers a result of inter-assay primer contamination with high concentration oligonucleotides? There are other (presumably unextendable) singleplex dimers with the RdRp probe as well.

The in-silico work is demonstrative of haste in design but should defer to wet performance seen in peer review journals which demonstrate unacceptable levels of promiscuous priming and false results that are not properly addressed in the Corman paper.

1)Muenchhoff et al writes

"A reduced sensitivity was noted for the original Charite' RdRp gene confirmatory protocol, which may have impacted the confirmation of some COVID-10 cases in the early weeks of the pandemic. The protocol needs to be amended to improve the sensitivity of the RdRP reaction."

https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7315722/
Drosten is an author on this paper that declares the need to replace the primers that were reviewed in 24 hours.

You cant have it both ways. If they are promiscuous primers and known to be leading to false positives or false negatives in the literature, then the Author of such disinformation has an obligation to correct record when peer review was clearly compromised.

2)Etievant et al

"The E Charité and N2 US CDC assays were positive for all specimens, including negative samples and negative controls (water). These false-positive results were explored (details below), but the
sensitivity of these assays was not further assessed."

"No false-positive results were obtained on clinical samples that tested negative for SARS-CoV-2 and/or positive for other viruses than SARS-CoV-2, except for E Charité and N2 US CDC, which were positive for all specimens.

Sensitivity was first assessed using SARS-CoV-2 cell culture supernatants. Using both specific SARS-CoV-2 (S) and non-specific (NS; detecting SARS-CoV-2, SARS-CoV, and bat-SARS-related CoVs) RdRp Charité assays"

Notice the term "non-specific" Charite' primers.

"Thus, the false-positive amplification obtained using E Charité
might be derived from a contamination (amplicon size at 121 bp) but could also be associated with an aspecific amplification (amplicon size at 84 bp)"

https://www.mdpi.com/2077-0383/9/6/1871

This paper stops evaluating the Charite' primer half way through the study due to performance issues.

3)Jung et al

"On the other hand, the RdRp_SARSr (Charité) set shows less effective amplification than the other two primer sets at all reaction temperatures including their recommended extension temperature (58 °C). Unexpected amplifications from NTC samples were observed with the RdRp_SARSr (Charité) set. The electrophoresis and melting curve analysis showed nonspecific amplification at lower positions (Lane 5, Figure S5b) and temperatures (Figure S5a) than the result of specific amplification with the RdRP_SARSr (Charité) set."

Evidence of Non Template controls amplifying in multiple peer reviewed journals will directly result in quarantine of non-infectious people and create a chain reaction of legal liability.

4)Gand et al.
"Interestingly, for Assay_2_RdRp-P2, similar false-positive results as obtained in our in silico study were obtained in the wet lab by Chan and colleagues, who detected SARS-CoV when using the probe P2 targeting the RdRp gene that is considered strictly specific to SARS-CoV-2 [30]"

"The sensitivity of Assay_2_RdRp-P2 (Charité) was already demonstrated in the wet lab to be lower than that of other assays investigated in this study"

These four manuscripts all point to flaws in these primers.

Christian Drosten is an author on one of these manuscripts that concludes they must be replaced!

The error prone primers still exist on the WHO website with a link to Eurosurveillance.

The liability of these erroneous methods leads back to Eurosurveillance and once they have been informed of the issue, we are confident they will do the right thing and retract the paper. A failure to retract the paper absorbs the liability as opposed to placing that liability onto the source of the deception; the authors themselves.

Citations are contained in this thread.
https://t.co/2St1wmWLAH?amp=1

Reply

1. **Peter Barnum** says:
   December 4, 2020 at 5:57 am

   This is not an answer to my question, it's a repetition of his twitter thread, which is worthy of its own discussion. I asked why in your report there is a primer dimer reported between two unrelated primers. None of the above mentioned papers said anything about multiplexing, nor does the original Corman-Drosten paper. For all I know right now, this is purely your assumption! And you still should have mentioned that this primer dimer only becomes relevant in multiplex assays. At the very least your report shows the same lack of scientific rigour it criticises in the Drosten-Corman paper!

Reply

1. **Kevin McKernan** says:
December 4, 2020 at 6:15 am

There is an in-silica single-plex dimer on RdRp probe. This is reported in the peer reviewed literature as causing problems. Primer contamination happens and primer designs for population level screens take this into consideration, especially when the effort to do so is automated with 20 year old in-silico tools.

Reply

1. **Peter Barnum** says:
December 4, 2020 at 6:32 am

Yes, that is all correct and all I want is this to be stated in this cautionary manner. How do you get from this to, and I quote from your twitter, “We are quite clear, that they have no clue what their primers detect as the methods produce primer dimers.” Which of these primer dimers leads to the PCR just detecting anything? Most of them rather lead to lower sensitivity, as it is extensively described in literature…

2. **Peter Barnum** says:
December 4, 2020 at 6:54 am

And this is all still ignoring that even in the original paper they tested 297 clinical samples from patients infected with around 20 different viruses and all of them were negative. Assuming that they didn’t blatantly made that up (which would of course be an unprecedented scandal), this also doesn’t support the “It colcd detect anything” hypothesis….

Bobby says:
December 4, 2020 at 9:20 am

Quote is out of the CD-paper:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.3.2000045#html_fulltext

Using the E and RdRp gene assays, we tested a total of 297 clinical samples from patients with respiratory disease from the biobanks of five laboratories that provide diagnostic services (one in Germany, … (Table 2). In total, this testing yielded no false positive outcomes.In four individual test reactions, weak initial reactivity was seen but 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 but most probably to handling issues caused by the rapid introduction of new diagnostic tests and controls during this evaluation study.

Notice here:

The one sentence states "no false positive outcome". The next sentence states: "In four individual test reactions, weak initial reactivity was seen but they were negative upon retesting with the same assay".

The first sentence becomes irrelevant and wrong, the second sentence states 4 (!) false positives out of 310.

In my opinion this is the kind of "cautionary manner" that led to the current situation worldwide, contradictions and the all-of-a-sudden-scientific acceptance of those, embedded in "cautionary manners".

The truth here is:

Four in 'n=310' primary-tests => false positives = 1.3 %.

1) This is a direct alegre to the many reported cases of "false positives", then "negatives".

Further:

2) Christian Drosten publicly states that this is his "validation concerning cross-reactivity", we tend to see this as a pseudo-validation:

https://twitter.com/c_drosten/status/1309755692232904704

This is no validation, there are 4 false positives, it's clearly stated in the text accompanied to table 2 of the CD-paper. Further, table 2 does not state a) which "validated clinical sample with known virus" caused the false positive, b) which gene assay.

2) The editors of Eurosurveillance further state in their communications:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.21.2001035

Quote:

Our strategy during establishment was to use a synthetic target for the SARS-CoV-2 E gene assay, while validating amplification of a full virus genome RNA using the RdRp assay that is specific for both, SARS-CoV and SARS-CoV-2, with the latter not being available to us in the form of an isolate or clinical sample at the time. Based on experimental validation, it later turned out that the mismatched base pairs do not reduce RT-PCR sensitivity and are not to be seen as the reason for somewhat higher Ct values with the RdRp assay as compared to the E gene assay [3].

Notice here:

“Based on experimental validation, it later turned out that the mismatched base pairs do not reduce RT-PCR sensitivity …”

Again we have this “cautionary manner”: “The basic copies do not correspond to each other BUT that does NOT effect the sensitivity.”… Trust us.

Nonsense! The supplementary data & material of this correspondance does not confirm that, which is provided with this editor’s / author’s communication response.

3. **Peter Barnum** says:
December 4, 2020 at 9:36 am

Once again, you are not addressing my main question: Which primer dimers do you think justify your claim that this PCR just detects anything? And you are interpreting “false positives” in “weak initial reactivity”. I agree that this sentence woud justify questions, however it does not justify the claims you are making, i.e. that this PCR test is not suitable to detect SARS-CoV2.

And the fact that over the last months huge test numbers have resulted in extremely low numbers of positive tests (i.e. this Chinese study https://www.nature.com/articles/s41467-020-19802-w) counteracts your more general claim that this whole pandemic is only “present” due to false positive PCR tests. Even if this particular study gets retracted by the journal, this does not change anything about the situation regarding SARS-CoV2.

Bobby says:
December 4, 2020 at 11:30 am

1) And you think the chinese study uses the Corman-Drosten protocol?

2) If so, could you back up this claim with references stating that they are using the CD-protcol?

3) We all know, that chinese laboratories are using much better protocols, f.e. Zhu’s, which was not recommended by the WHO, even though it was submitted to them in January 2020.

4) Drosten refutes himself here:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.24.2001057

To quote Kevin Mckernan:

Muenchhoff et al. Same Journal

Conclusion:
RdRp needs to be pulled.

Drosten is an author. Same journal.

Can’t have it both ways.

The WHO still points to old and faulty primers and the first review clearly missed the error. We expect this to be withdrawn soon!

Everything else is irrelevant concerning your supposedly-not-answered-question in regards to our review report on the CD-protocol, and also the referenced nature article (chinese study): We know that they are not using the CD-protocol, so it is irrelevant for our review report, which focuses on the Corman et al. publication and nothing else. This is a fallacity in itself.

5) We know that most of the western hemisphere uses 2 confirmatory assays. We know that China uses much better protocols (see point 3) than the CD-protocol. We know that Taiwan even uses 3 confirmatory assays – as everyone should (also compare the specific sections on this in our review report). AND we know that Thailand uses two genes when arriving there, and when initially tested positive, then another 5 genes are used for confirmation to decide if the person is indeed positive or not, thus nearly no cases in Thailand.

So in summary I’d say, your question is heavily irrelevant in regards to our review report because it sets the scope outside the Corman-Drosten Protocol review (it’s not so hard to stay on topic, our review report sets the framework).

Bobby says:
December 4, 2020 at 11:38 am

Addendum to your question: copypaste “Answer” by Prof. Dr. Ulrike Kämmerer:

https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.24.2001057

All labs have used ONE sample which was comprised of several extractions of RNA from ONE stool sample as positive control for SARS-CoV-2.

I wonder why they had not used the RNA of (well characterized?) SARS-CoV-2 isolates, like the one which is sold by the Charite (https://www.european-virus-archive.com/virus/human-2019-ncov-isolate). and labelled to be isolated from a Munich patient in Janary 29….

The article was submitted in May – so they should have access to not only this isolate but to many different isolates from all over the world as real positive controls. Further, no negative control and no control samples with the RNA of other Coronaviruses were shown.

So its a publication on the detection of RNA from one Patient (and unusual: stool sample – why not from respiratory samples?) in different dilutions....

And not supporting anything about specificity but only sensitivity of the dilution series of this one specific sample.

2. **Peter Barnum** says:
December 4, 2020 at 6:09 am

Aand: Thanks for the pointer, must have overlooked that – my bad!

Reply

67. **NN** says:
December 2, 2020 at 12:50 pm

This website here is nonsense. See here for the reasons why: https://twitter.com/BMauschen/status/1333468806203793411

Reply

 **Bobby** says:
December 2, 2020 at 1:18 pm

We have answered Mäuschen extensively on her confusing thread, and also invited her to participate at the discussion directly here with all the authors. She didn't follow the invitation until yet. All her points are directly refuted and thoroughly debunked several times in her thread, it seems like she has no clue about the things she claims to have any kind of expertise. This thread is a PR think tank campaign by Christian Drosten & Co and we are quite disappointed that Christian Drosten & Victor Corman need paid shills (who don't know anything about the stuff that is written on their scripts) to address our 10 concerns, we would have expected more scientific integrity and backbone to be honest.

Reply

68. **Tony** says:
December 2, 2020 at 2:37 pm

I would like to share some comments or questions:
1... nm means "nanometer" ....
This is a typo, no more and no less, and any lab technician will recognise it immediately.
2."First, a positive test for the RNA molecules described in the Corman-Drosten paper cannot be equated to "infection with a virus"
Where else would the RNA come from, if not through replication of the virus in the host. Could high RNA concentrations be caused by "contamination"?
3."...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…"
Detection of the full-length construct would be too costly, time demanding and therefore counterproductive. If you consider the PCR assay unsuitable for diagnosis, what about PCR-based liquid biopsies?
4."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) …"
The test does not work, because it was not originally validated with the isolated virus? Sounds weird to me.
5."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."
So far I remember, there were a lot of positive and negative controls included.(?)

6. "The first sentence of this excerpt is clear evidence that the PCR test described in the Corman-Drosten paper generates false positives"
„however they were negative upon retesting with the same assay". Positives will be retested anyway, even by amplification of another gene, isn't is?

Reply

69. **Stringer** says:
December 2, 2020 at 3:02 pm

Scenario:

1. SARS-Cov2 effectively does not exist except as a theoretical construction in a computer
2. It is part of the pandemic simulation called "Event 201" which aims at traumatizing and controlling the population and enabling public-private partnerships to plunder cash and resources on a massive scale
3. There is no preparation of the virus available in any lab worldwide in amounts sufficient to perform independent determinations of its identity, defined as a complete elucidation of the viral structure and characterization of its biological activity
4. There is no preparation of the virus available in any lab worldwide in amounts sufficient for use as a laboratory standard
5. No such virus has been isolated from any human dead or alive in sufficient quantities to perform a complete determination of identity (structure and biological activity) in order to compare it with a standard
6. Therefore the current PCR test is "detecting" RNA fragments from common corona viruses that have been around for years
7. Even if the test is substantially improved, it will be chasing a phantom however many sequences may be simultanously targeted
8. There is no indication of the clinical utility of the PCR test except to tyrannize the population by enforcing quarantine and other punishment
9. The clinical utility of the PCR test for the tested persons is zero, otherwise such tests would have been in use for at least a decade to support diagnosis and therapy of influenza or heavy cold cases.

Factual and logical rebuttals are cordially invited.

Reply

1. **Joerg** says:

December 2, 2020 at 3:50 pm

Yes it is a (your unreal) szenario!
1) (non existence) Ask ill people or read cases, look to excess mortality charts (besides Germany)
2) (conspiracy) Honestly, do you feel traumatized & controlled? Actually, we are far through the tunnel already. Fear, ignorance, incompetence, incapacity are enough to explain the most.
3) (no big amounts of virus preps) Can't comment on this one (no expert).
4) (no lab standard available) Could be synthesized/PCR-amplified of infectious material?
5) similar as 3) see 3)
6) (common corona virus detected) Why then is there an excess mortality of positive tested patients?
7) (there is no improved test) That's what I'm asking. Why not? There are specific genes and discriminating sequences (stable enough/without mutation pressure) compared to common corona viruses?
8) (no clinical advantage) Yes. The tests are established to monitor the spreading, to shelter the healthcare system. Epidemiological reasons! (NOT clinical)
9) similar as 8) see 8)
Kind regards Joerg

Reply

1. **Dr Solomon** says:
December 3, 2020 at 2:50 am

There is no excess mortality in UK where I practice as a doctor.
The numbers presented (59K) also include those thousands who were literally murdered in their care homes by Boris Johnson Govt as they were denied access to hospital.It also includes those who died months later from other causes and had tested positive (false positive) earlier.It also includes thousands of people who died in their homes due to heart attacks, strokes, infections and cancers because they were frightened to death by BBC propaganda and did not come to hospitals! So are you still surprised by slightly increased numbers of death this year ??
Doctors think and Robots don't !
Kind Regards

Reply

1. **Joerg** says:
December 3, 2020 at 9:34 am

Hello Dr. Solomon,

please go to this site, they track the excess mortalities for Europe:
http://www.euromomo.eu/graphs-and-maps
Please, scroll down to the UK graph, there is a tremendous peak in excess mortality in the calendar week 15 2020 (z-score 36).
Imagine, if you say "all these data (for other countries too) are a concerted fraud", you better bring up proofs beside selective, specific, personal

observations?
Why is the „fraud“ so naïve, leaving out countries as Germany (there is indeed no excess mortality) making a “conspiracy theory” more difficult?
Who should have ordered this fraud? How could it be concerted among different countries which are fighting each other on the level of EU policies?
The answer is: VERY, VERY PROBABLY THE GRAPHS PRESENTED ARE NOT WITHOUT MISTAKES BUT IN TOTAL THEY ARE THE BEST ESTIMATE OF THE TRUTH WE HAVE?!

Off course, one could and should earnestly discuss all the governmental actions according the “pandemic” (besides it is more or less severe).
But it is to easy to borrow “simple” answers for complex questions! This is typical human, but behold and think.

Thanks for your service as a doctor, much power and strength for your duty.
Joerg

Reply

1. **Thomas Ellenberg** says:
   December 3, 2020 at 9:16 pm

   “Please, scroll down to the UK graph, there is a tremendous peak in excess mortality in the calendar week 15 2020.”

   A virus is definitely not responsible for such a sharp peak. The cause was massive, experimental testing of potentially lethal dosages of hydroxochloroquin which was initiated by the WHO. That’s why you see this peak in multiple countries at the same time which, again, can’t be caused by a virus. And you allready mentioned that it didn’t happen in Germany. Well, it used reasonable dosages.
   https://www.youtube.com/watch?v=0JcVglSdQ-c

2. **Joerg** says:
   December 4, 2020 at 10:56 am

   @Thomas Ellenberg
   That does not come temporally there!

   Better check the facts and don’t blindly believe what you pick up on the Internet.
   https://www.nature.com/articles/s41419-020-2721-8

   It was not until March 28 that hydroxychloroquine (HCQ) was briefly introduced by the U.S. Food and Drug Administration (FDA).
   There were already many patients in the hospitals, do you really think that all of them had administered HCQ from 29th March (week 14) and in week 15 (peak) so many were dead? What about all the people who died in nursing homes or at home? Do you think they all received HCQ

from their GPs as of 29 March?
If you have verifiable sources of it, give it to me.

Of course, it is true that many suboptimal treatments took place in March/Apr, but thanks to God, the mortality rate has decreased over time. A merit of the research, the doctors, the nursing staff.
As I said, it is easy to discuss whether many restrictions on the part of politics are exaggerated.
Nevertheless, don't get involved in every witch-hunt! Please consider the scientifically verifiable facts, not just opinions on YouTube.

Kind regards Joerg

3. **Thomas Ellenberg** says:
December 4, 2020 at 9:40 pm

"Better check the facts and don't blindly believe what you pick up on the Internet."

Better try to avoid irrelevant ad hominem spam and other fallacies.

"There were already many patients in the hospitals, do you really think that all of them had administered HCQ from 29th March (week 14) and in week 15 (peak) so many were dead?"

HCQ was the MAIN component of mal pratice. Using ventilators without medical necessety or using combos of immunosuppressive drugs were amongst many others. So what I'm saying is that malpractice was the reason for the sharpness of these peaks. And it happened simultaneously in multiple contries, especially in those with lots of deaths in Europe, but not in all which shows that this was man made. Even within Switzerland there was a difference between the mortality in the German, Italian or French speaking region and excess mortality was only found in the latter two. I highly doubt that a virus prefers killing French or Italian hosts.

I linked a Youtube video with Claus Koehnlein who also co-authored this article:
https://www.rubikon.news/artikel/die-medikamenten-tragodie

2. **A. Berglund** says:
December 3, 2020 at 5:15 pm

1)You cannot prove that the "ill people" and "cases" are infected with "SARS-COv2". The "diagnostic test" for RNA fragments is useless. It is a random number generator on the set (0,1).

2)"through the tunnel"–I'm sure the proprietors of hotels, restaurants, fitness and yoga studios and the travel industry who have lost up to 80% of their revenue compared to 2019, and the workers who have lost their jobs, will derive great comfort from those words. You must have a well-paid government job.

(6)and (8)the diagnostic test is useless for any purpose

Reply

70. **roland brautigam** says:
December 2, 2020 at 4:39 pm

Hi Bobby – assume you have seen that Eurosurveillance published this in 2012. Note that Ab Osterhaus is one of the authors. The devil himself!

https://www.eurosurveillance.org/content/10.2807/ese.17.49.20334-en

Reply

71. **tim** says:
December 2, 2020 at 5:15 pm

it's revolution time…

Reply

72. **Pierre Lutgen IFBV-Belgerb** says:
December 2, 2020 at 8:48 pm

The same questionable PCR testing is used to hide the inadequacies and failures of ACT (Coartem, Coarsucam, Artequick) malaria therapy

Reply

73. **Rik Breuer** says:
December 2, 2020 at 8:51 pm

I would go one step further and question the validity of the "finding" of the virus itself.

I read the paper regarding the Chinese scientists in Wuhan taking a sample from "one" sick patient and "identifying" the new virus SARS-CoV-2. They didn't isolate the virus (that never happened anyway), they didn't fulfill Koch's postulates and without a Gold Standard they couldn't prove that this "virus" is causing an flu like illness which can't be clinical diagnosed.

I would say, this whole Covid19-pandemic is a scam from beginning to end.

I have looked up excess mortality in US and Germany. There is none.

Reply

    1. **Kevin Corbett** says:
    December 2, 2020 at 11:36 pm

    I agree with you.

    Reply

74. **Terrence Joseph Bennett** says:

December 2, 2020 at 10:07 pm

Thank you for doing science!
Shame on those who prefer Crapitalism to truth

Reply

75. **heusenbeck** says:
December 3, 2020 at 2:21 am

Why is "A) BACKGROUND" a major concern with the corman drosten paper?

"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?"

To quote Pieter Burger from another paper he released in april 2020:

"It was common knowledge that some strain of coronavirus-sooner or later-was going to cause a pandemic. It was known since the SARS-CoV-outbreak in 2003. In 2013 and 2015, the world was informed that a variant of SARS-CoV in bats was emerging as a threat for humans."
[https://www.researchgate.net/publication/341120750_A_SARS-like_Coronavirus_was_Expected_but_nothing_was_done_to_be_Prepared]

Like any virologist Drosten warned about the threat of emerging infectious diseases and as we know, he co-discovered SARS-CoV1, developed the first diagnostic test and contributed to research on MERS early on. So i dont know what the implification of your "major concern A)" is. Do you expect him or any scientist working in this field, with the necessary resources, to sit back and check google statistics till death rate is alarming?

Reply

76. **Dr Solomon** says:
December 3, 2020 at 2:39 am

Dear team of Great Scientists,

Well done. You would be remembered in human history as those who chose to speak up against those who chose to sell their conscience to Pfizer, Astra-zeneca and Bill Gates.

I am a physician in UK and I see this fake and false positives on daily basis where literally hundreds of patients are being isolated and traumatized just because their fake PCR test has detected some unknown bits of unknown RNA despite the fact that none of them would have any symptoms whatsoever !This madness in being practiced on daily basis across European hospitals under the name of Science(Fake Science)and worst of all, under the auspices of doctors who took oath of not harming their patients and defending them against the interests of the Mafia of Bill Gates and likes.

I understand these are unique and hard times in human history where the forces of big Pharma, Large banks and huge corporations are hell bent on enslaving us and taking away the very last bits of our independence and humanly existence as an extremely endangered specie.

The question is how can masses around the globe resist this mania ?

Wouldn't it be better if doctors and nurses around the globe came together under one umbrella with a view to give the common men their right to know the truth and help them fight back against these demons ?

I strongly believe it is the sole responsibility of health professionals around the globe to protect the people against this wave of sheer medical barbarianism , neo-slavery and worst fraud of human history. Nobody wants to be part of this new SS Medical Corps of Pfizer and others (Angels of Death) who are committing the most heinous crimes against humanity. What Bill Gates, Mafia of World Economic Forum and likes are suggesting looks very similar to what Hitler and his SS Medical Corps were doing seven decades ago !

I look forward to your thoughts on this. I bet Bill Gates will not be happy to hear this for he would prefer Robots to thinking doctors which is perhaps his next agenda!
Before we are all robotized in near future, it is time to wake up. A global Consortium of Doctors and Nurses backed by the people is the solution.

Together, We Can Make A DIFFERENCE!
IT IS NOW OR NEVER…………

Reply

77. **Busu** says:
December 3, 2020 at 6:16 am

Thank you for this important job!
What are your next steps? In Germany there are no reports about this study, I don't know are there to prevent this study.

Reply

78. **Franz** says:
December 3, 2020 at 7:28 am

Thanks for your huge effort giving a review on this paper. Would be great to get some more information about practical work of authors done on detection of SARS-CoV-2.

Reply

79. **Joerg** says:
December 3, 2020 at 9:11 am

Hello,

in the meanwhile I got answers from a molecular lab expert involved in Covid19 research & diagnostics:

Overall:
– One should rather honor that within days and largely based on alignments of SARS-like viruses such well-working PCR assays have been designed, one of which (E gene) is still the reliable backbone of diagnostics

Technically:
– Why discuss annealing temperatures when you can show experimentally that these PCRs

work?
– Why discuss wobble bases when the RdRP PCR is demonstrably specific and sensitive (statement from practice: we had more than once a false base in the primer, which made a specific PCR-assay better instead of worse)
– Why discuss the 'right' amount of primers? If the reverse RdRP primer is too weak (too short) and contains wobble bases, then it is only logical to use more of them [higher concentration]
-> PCR IS MUCH MORE EMPIRICAL THAN DESIGN THEORY!
Auf deutsch: "Entscheidend ist, was hinten rauskommt!"

Sensitivity & Specificity:
– In the January publication sensitivity (5 copies) and specificity (other cold pathogens do not make a signal) were determined experimentally.
– What is a diagnostic PCR? To find negative samples negative and find positive samples. These three PCR assays do this reliably.

Improvements since Jan:
– For RdRP an interaction with human DNA had been overlooked. The RdRP assay has different primers since March.

What has been researched since Jan?
– Well over 100,000 viruses have been sequenced since January and they are virtually all identical.
– The diagnostic regions were rarely affected by mutations (once Roche cobas and once CDC).

Conclusion:
– Without 'new' results (sequences) there are few needs for new assays [because the current ones work]

Kind regards Joerg

Reply

1. **Max** says:
December 3, 2020 at 9:50 am

same her – much fuzz about something that doesn't matter in practice. To the "reviewers":

.) you criticise that many of the steps of validation have been done with virtual data, but you never tried the assay on the bench. You have just gone through it "virtually". You had months of time to proof that the assay does not work (in the lab, on the bench!), but you did not even try to do it.

.) why is it important for you to discriminate between a virus fragment and whole virus? how should it get there if not via infection? There are no virus-pcr-detection systems, that detect the "whole virus", it's always a specific fragment you are searching for.

.) There are no Wobbly-positions in the 2019-nCoV specific primer.

.) there is no SOP and this could be improved, yes. but drosten gives information about sample preparation, primer-sequences, limits of detection and setup for PCR.

Any routine lab can handle this information and setup their tests.

.) The cT value already has been corrected by drosten in an interview to a recommendation to cT ~30. But as this differs between labs, it should not be taken too precise, but validated by the labs. much more important are control samples, which are taken by serious labs anyway – positive virus sample, positive human DNA-sample, negative load,..

stay safe!

Reply

Bobby says:
December 3, 2020 at 11:01 am

REPLY FROM THE AUTHORS (DR. BORGER):"ANSWERS":

QUOTE:
.) you criticise that many of the steps of validation have been done with virtual data, but you never tried the assay on the bench. You have just gone through it "virtually". You had months of time to proof that the assay does not work (in the lab, on the bench!), but you did not even try to do it.

ANSWER:
We have now several labs worldwide, which report that the C-D test does not work in their labs and generates false positive results in different PCR machines. So this test cannot be sold as the golden standard.

QUOTE:
.) why is it important for you to discriminate between a virus fragment and whole virus? how should it get there if not via infection? There are no virus-pcr-detection systems, that detect the "whole virus", it's always a specific fragment you are searching for.

ANSWER:
Bad news for virus diagnostics. PCR virus diagnostics should only be to support differential diagnosis to exclude other virus diseases. It should never be used as a screening device, since virus-parts are inhaled all day long and trapped in the mucous of nose and lungs, exactly there were the samples are taken from.

QUOTE:
.) There are no Wobbly-positions in the 2019-nCoV specific primer.
.) there is no SOP and this could be improved, yes. but drosten gives information about sample preparation, primer-sequences, limits of detection and setup for PCR. Any routine lab can handle this information and setup their tests.

ANSWER:
Bad science and even worse for diagnotics. There must be an online SOP, which must be used in all labs, germany-wide, world wide. And the SOP should be online update when required.

QUOTE:
.) The cT value already has been corrected by drosten in an interview to a recommendation to cT ~30. But as this differs between labs, it should not be

taken too precise, but validated by the labs. much more important are control samples, which are taken by serious labs anyway – positive virus sample, positive human DNA-sample, negative load.

ANSWERS:
When Ct is different Germanwide, worldwide, nothing can be compared. This is very bad science.

QUOTE:
stay safe!

ANSWER:
Stay free!

Reply

1. **Max** says:
December 3, 2020 at 3:20 pm

1) so you did not try to test the assay. just used "virtual data" and personal communication. why dont they have controls for false positive test – its no rocket science.

2) yes, virus diagnostics should only be one part and this is what drostens lab is saying. its not a corona-test, but a procedure they claim to be done! But no, there are no "dead" virus particles free floating, just happen to infect people. and no, even if you catch up one of these, they will not be tested positive! they need to reproduce 2-3 days until pcr-test will recognise them (thats wy pcr tests are false negative the first two days 😉

3) this can be enhanced, yes, but does not make the test itself bad. every trained lab-worker handling with pcr knows what to do, when getting drostens publication!

4.) well, this is fact with every PCR, and also every other (diagnostic) test. unless you use the exact same reagents (including LOTs) and the same equipment (and you can never do this in two different labs) you will have differences. Thats what trained lab-workers are here and you must not let beginners/student or simply untrained personnel do the diagnostics! even in the same lab you have to re-evaluate and re-validate your SOPs from time to time, also if you do not change anything. thats science. thats good science. relying on SOPs someone else made up with different reagents (included buffer solutions from different companies etc) is bad science.

Reply

1. **Lorenzo** says:
December 5, 2020 at 6:17 pm

Max, how can a lab practically validate its results? You talk about control samples, I assume a _certain_ positive and a _certain_ negative, how can a lab acquire these? Regarding the samples I'm thinking about actual swabs material with a mix of human and multiple bacterial and viral genomes. I've read papers where water with just individual viruses was used as a negative, it seems quite different from the real world situation.

Reply

Bobby says:
December 3, 2020 at 10:59 am

REPLY FROM THE AUTHORS (DR. BORGER): "ANSWERS":

QUOTE:
Overall:
– One should rather honor that within days and largely based on alignments of SARS-like viruses such well-working PCR assays have been designed, one of which (E gene) is still the reliable backbone of diagnostics

ANSWER:
Indeed, but wouldn't it be nice to know what it is detecting? It was not molecularly validated. It may detect any coronavirus and probably other viruses as well.

QUOTE:
Technically:
– Why discuss annealing temperatures when you can show experimentally that these PCRs work?
– Why discuss wobble bases when the RdRP PCR is demonstrably specific and sensitive (statement from practice: we had more than once a false base in the primer, which made a specific PCR-assay better instead of worse)
– Why discuss the 'right' amount of primers? If the reverse RdRP primer is too weak (too short) and contains wobble bases, then it is only logical to use more of them [higher concentration]
-> PCR IS MUCH MORE EMPIRICAL THAN DESIGN THEORY!
Auf deutsch: "Entscheidend ist, was hinten rauskommt!"

ANSWER:
We agree, but the Test is designed so sloppy, we simply don't know what positive results mean.

QUOTE:
Sensitivity & Specificity:
– In the January publication sensitivity (5 copies) and specificity (other cold pathogens do not make a signal) were determined experimentally.
– What is a diagnostic PCR? To find negative samples negative and find positive samples. These three PCR assays do this reliably.

ANSWER:
No, there is nothing in the test to exclude other viruses.

QUOTE:
Improvements since Jan:...

ANSWER:
Irrelevant for our external review report.

QUOTE:
What has been researched since Jan?
– Well over 100,000 viruses have been sequenced since January and they are virtually all identical.

ANSWER:
He means "puzzled together from 200 bp pieces" using a prespecified SARS-CoV-2 template.

QUOTE:
– The diagnostic regions were rarely affected by mutations (once Roche cobas and once CDC).

ANSWER:
There are now over 30'000 difference Sequences collected, including indels, in glue: http://cov-glue.cvr.gla.ac.uk/#/home So, if 100,000 sequences have been collected, 1 in 3 is different.

QUOTE:
Conclusion:
– Without 'new' results (sequences) there are few needs for new assays [because the current ones work]

ANSWER:
We have now reports from all over the world that the PCR test does not work as described in the Corma-Drosten paper. Positive often even depends on the instruments used. This test should not be propagated as the golden standard, because it is not golden.

Reply

 **Bobby** says:
December 3, 2020 at 11:11 am

REPLY BY THE AUTHORS (PROF.DR. KÄMMERER): "ANSWERS":

ma additional comments:

QUOTE:
– One should rather honor that within days and largely based on alignments of SARS-like viruses such well-working PCR assays have been
designed, one of which (E gene) is still the reliable backbone of diagnostics

ANSWER (ADDITIONALLY TO DR.BORGER'S ANSWER):
There was NOR REAL nCoV2019 positive control in the test validation.

QUOTE:
Technically:
– Why discuss annealing temperatures when you can show experimentally that these PCRs work?
– Why discuss wobble bases when the RdRP PCR is demonstrably specific and sensitive (statement from practice: we had more than once a false base in the primer, which made a specific PCR-assay better instead of worse)

ANSWER:
This is not a good laboratory practice – normally you can create an PCR that is optimized for the gen target to be detected, especially in a case where sequences are available in the Genbank (or viruses GISAID). The Chinese CDC managed to fulfill ths aspect for both the primer pairs and probes for their very early PCR, so why did the authors of the cormandrosten PCR publication such a weak PCR compared to the Chinese scientists?

QUOTE:
Why discuss the 'right' amount of primers? If the reverse RdRP primer is too weak (too short) and contains wobble bases, then it is only logical to use more of them [higher concentration]

ANSWER:
There is no need to include wobble bases in a PCR when the correct gene sequence is known (se figure 2 in the Cormandrosten paper). One can easily select a primer pair that fits and can be used at low concentrations with the optimal melting temperature around 60°C by one of the many good database "Primer design" tools . In daily laboratory practice you create 2-3 different primer pairs for the same target, order them and then then you test them in the lab with correct positive and negative controls to figure out which pair works best. After further validation with "real" samples (tissue, swabs, cell culture, etc. you can publish it. Well, maybe this it common practice for "normal" labs
>>>> only, maybe not for TOP-virology labs....<<<<<

Reply

1. **Kevin McKernan** says:
December 4, 2020 at 5:01 pm

Please see my comments addressing this for other questions proposed on this thread. There is no need for us to perform wet experiments when 4 peer reviewed articles are provided with wet evidence of false positives generated and a recommendation to eliminate the RdRp primers. One of the authors of a paper suggesting the RdRp assay be eliminated is published in Eurosurveillance with Drosten as an author (Muenchhoff et al). You can't have it both ways. 24 hour review had bad primers and the evidence comes from a paper with Drosten as a contributing author. It is his responsibility to remove the disinformation that he published before more false positives wreck peoples lives.

Reply

80. **wilhelm Lehberger** says:
December 3, 2020 at 1:34 pm

Es sollte selbstverständlich sein, dass im wissenschaftlichen Bereich und gemäß dem Anspruch einer demokratischen Gesellschaft Positionen ausgetauscht, geprüft und insbesondere im Falle derart gravierender Auswirkungen öffentlich erörtert werden können. Dies findet nicht statt und findet sich auch hier nur ansatzweise wieder. Besorgte Nachfragen werden ignoriert und zu oft treten die übelsten menschlichen Eigenschaften zutage – denunzieren und diffamieren. Wenn also ein Ehepaar vor einer Reise Antigentests und in zeitlichem Abstand einen PCR-Schnelltest beim Hausarzt vornimmt, vorsorglich 3 Wochen private Kontakte ausschließt und der PCR-Test dann 1x negativ + 1x positiv ausfällt, dann ist das angesichts der hohen Infektionsgefahr nicht zu erklären. Das ist kein Einzelfall und nach dem Informationsstand nicht zu erklären. Es traten auch in der folgenden Quarantäne (die das Gesundheitsamt erst nach Ablauf und 4 Wochen nach dem Test anordnete) keine spezifischen Symptome auf. Was heißt das nun? Wurde man nun infiziert oder nicht? Wenn ja, ist man dann "geheilt" oder "immun"? Bringt dazu ein erneuter Antigentest Erkenntnisse? War hingegen der Test falsch, bedeutet das ca.320 € für die Tests und 3.500 € Stornokosten (die Versicherungsbedingungen wurden zum 1.9. angepasst). Faktisch ist damit die Reisefreiheit auf unbestimmte Zeit aufgehoben, weil sie keinen "richtigen" PCR-Test innerhalb von 24 Std. nicht bekommen. Die "offizielle" Antwort wäre sicher, man solle ja nicht verreisen (gilt nicht für Pendler u. Geschäftsleute). Da muss man sich über Proteste nicht wundern und schon sind wir wieder (auch hier) bei Rechtsradikalen und Verschwörungstheoretikern. Da empfehle ich doch den Blick über den linken Zaun, da gibt es erhebliche Zweifel bis zum Protestaufruf. Und wenn Klaus Schwab und das Weltwirtschaftforum Verschwörungstheorien befördern (wie das in einem Kommentar anklang),zu dem u.a. Minister und Staatschefs anreisen, dann sollte das die Politik klarstellen. Dazu die alte JUSO-Frage: Wem nützt es? (Und wer zahlt es?)

Reply

1. **V. Scholz** says:
December 3, 2020 at 4:16 pm

Dieser Test ist ein Zufallszahlgenerator.

RA Fuellmich redet nicht umsonst vom "vorsätzlichen sittenwidrigen Betrug".

Reply

81. **Oscar Drinnon** says:
December 3, 2020 at 4:33 pm

The use of bald-faced lies about an epidemic to conceal a political agenda was exemplified as early as 1968 in Stanely Kubrick's famous film "2001, A space odyssey".

The android-like bureaucrat "Dr. Heywood Floyd" informs moon base scientists that an epidemic is being used as a cover story for the news blackout after the discovery of the black monolith. He further informs them that they will have to sign a written oath of secrecy:

'– https://www.youtube.com/watch?v=PdbhZBpqNPM –'

Reply

82. **Michael** says:
December 3, 2020 at 5:19 pm

Eurosurveillance has reacted:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.48.2012031

Let's hope science wins!

Reply

1. **Gyarmati** says:
December 3, 2020 at 6:50 pm

No way. They will reject the above paper and the retraction demand. The narrative cannot be threatened.

Reply

83. **Frank Visser** says:
December 3, 2020 at 5:41 pm

This contradicts your story that the C/D PCR is defective, is completely aspecific and can't even work:

Comparative Performance of SARS-CoV-2 Detection Assays Using Seven Different Primer-Probe Sets and One Assay Kit

"We found that the most sensitive assays were those that used the E-gene primer-probe set described by Corman et al. (V. M. Corman, O. Landt, M. Kaiser, R. Molenkamp, et al., Euro Surveill 25:2000045, 2020, https://doi.org/10.2807/1560-7917.ES.2020.25.3.2000045) and the N2 set developed by the CDC (Division of Viral Diseases, Centers for Disease Control and Prevention, 2020, https://www.cdc.gov/coronavirus/2019-ncov/downloads/rt-pcr-panel-primer-probes.pdf). All assays tested were found to be highly specific for SARS-CoV-2, with no cross-reactivity with other respiratory viruses observed in our analyses regardless of the primer-probe set or kit used."

Reply

Bobby says:
December 3, 2020 at 5:47 pm

We also have this editorial note, in my honest opinion, that doesn't sound plausible at all:
https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.21.2001035

Quote:
Our strategy during establishment was to use a synthetic target for the SARS-CoV-2 E gene assay, while validating amplification of a full virus genome RNA using the RdRp assay that is specific for both, SARS-CoV and SARS-CoV-2, with the latter not being available to us in the form of an isolate or clinical sample at the time. Based on experimental validation, it later turned out that the mismatched base pairs do not reduce RT-PCR sensitivity and are not to be seen as the reason for somewhat higher Ct values with the RdRp assay as compared to the E gene assay [3]

"More mismatches? No problem, test works, we assure you".

Reply

Bobby says:
December 3, 2020 at 5:59 pm

Sensitivity is not specifity. A lot of false positives increase sensitifity but decrease specificity.

Reply

84. **Ale vd sluis** says:
December 3, 2020 at 11:59 pm

nu maar weer hopen of niet, dat u uw inteletuaeel vermogen ten positive weet te aan te wenden. ik begrijp de frustratie maar denk echt dat er betere manieren zijn om uw gelijk aan t tonen als dat er al uberhaupt al toe doet.Kom op peter, waar blijft die toegevooegde waarde

Reply

1. **Robert** says:
December 4, 2020 at 8:11 pm

Heeft u bij Peter in het dorp gewoond? Aan uw Nederlands te lezen heeft u in ieder geval niet op dezelfde school gezeten. Mag ik u vragen wat u zelf vindt van de toegevoegde waarde van uw opmerkingen in een verder behoorlijk volwassen discussie?

Reply

85. **Emalsen** says:
December 4, 2020 at 12:22 am

No mention that the protocol in question was updated or of any further development in testing. Instead the authors, in comments above, made clear that their criticism aims solely at the original paper from nearly a year ago.
In wich scale this specific protocol was or might still being used, the report unfortunately does not investigate. So any impact of misdiagnosis, even if they prove this old protocol eventually somehow guilty, stays unfounded. I wonder what they try to gain here?

Reply

Bobby says:
December 4, 2020 at 5:43 am

You are missing something substantial, and I suppose "on purpose", the Eurosurveillance CD-paper has an Addendum, the Addendum shows the changes made:

Quote:

*Authors' correction

The sentence As at 20 January 2020, 282 laboratory-confirmed human cases have been notified to WHO was originally published with a wrong date (As at 20 January 2019…). This mistake was corrected on 8 April 2020.

On 29 July 2020 the correct affiliation of Marco Kaiser was added and the remaining affiliations were renumbered.

** AddendumGo to section...

The Conflict of interest section was updated on 29 July 2020.

So your comment is a totally wrong & a weak lie – I suppose on purpose. Scroll down: https://www.eurosurveillance.org/content/10.2807/1560-7917.ES.2020.25.48.2012031#html_fulltext

P.S.: Announced changes via Christian Drosten's podcast don't count. We want it in written official form.

Reply

1. **Rob** says:
December 4, 2020 at 8:50 am

That's an issue that could be resolved by one short email from every test kit's manufacturer, if it's not already included in their literature. We already know the cyclic thresholds are way too high for all the tests, I suspect they probably contain many if not most or all of the issues raised here – easy enough to find out though.

Reply

86.  **Ale vd sluis** says:
December 4, 2020 at 1:12 am

peter , moast even dyn moderater bettere instrukties hjaan ,

Reply

87. **Willy Schmid** says:
December 4, 2020 at 8:44 am

Please try to stop testing worldwide contacting the right persons/organisations who are ableto do this. By now, almost everybody knows that the PCR-test is not good at all. But in all countries, they continue to test, making more lockdowns. Now testing is even made with a quick test, which is less precise than the PCR-test according to the manufacturer Roche. Thanks.

Reply

88. **Y. Gielens** says:
December 4, 2020 at 9:01 am

I have not seen an analysis of the effects of variability in the sampling procedure, sample storage and sample extraction on the test results. (These are all steps prior to the test proper).

Examples of variables:
–Design and material of swabs. Are they fit for purpose? Have they been tested for contaminants that can cause false-positives? Is the production process controlled?

–Nature and amount of sample removed onto the swab from the oral or nasal cavity: (a) what matrix is actually sampled? Mucus? Water? Cell layers? (b) How much sample is removed, measured in microliters (fluid) or micrograms (solid)?

–What is the extraction variability from the matrix and from swab to swab?

–Effect of sampling site (oral or nasal). Is one targeted or both? Why?

–Stability of the molecular species to be anayzed (oligonicelotides?) in the sample after collection and during transport: temperature, mechanical stress?

–Efficiency of extraction from the sample matrix/swab of the molecular entities(oligonucleotides) to be analyzed ?

Have all of these factors been tested, for example by spike/recovery experiments?

If two subjects "A" and "B" have the same amounts of RNA at the nasal/oral sampling sites, but subject "A" tests positive and subject "B" does not, the discrepancy could be due to variability in any of the above factors. Just one example: removing double the amount of sample material onto the swab from the nose and throat of A compared to B would suffice.

These are just technical points apart from the main issue of whether the molecules being analyzed have amy clinical relevance. But as long as (incredibly)such tests are still being performed, they have to be considered.

Reply

89. **Ellis Mulder** says:
December 4, 2020 at 9:06 am

Thank you so much! Anyone should know this! I'm so proud of you all!

Reply

90. **Y. Gielens** says:
December 4, 2020 at 9:11 am

A further question that I have not seen addressed:

The test apparently produces a binary yes/no output–"positive" or "negative".

But what is the actual physical signal readout from the instrument? Subjective visual? Photometric? fluorimetric? Which wavelengths?

Such instrument signals are normally continuous variables. What criteria are used to convert them into the binary ("positive / negative") readout and what are their statistical properties?

What data reduction techniques are used (standard curves?) Confidence intervals?

Has an error analysis of the entire test procedure from sampling inn the subject to instrument readout and data analysis been performed–e.g. within and between-run imprecision, inaccuracy, variance components attributable to each step in the method?

I find the concept of a yes/no readout in clinical chemistry and clinical pharmacology rather fantastic –almost pseudo-science.

It’s like if the doctor says: “Well, we did a thyroid workup on your blood and the result is positive. However, the cholesterol came out negative”.

Reply

91. **Christl Meyer** says:
December 4, 2020 at 11:00 am

Thank you. It´s the same with testing for “HIV”.
I am fighting since more than 20 years for truth.
https://www.academia.edu/11649973/GENE_OR_VIRUS_IN_HEALTH_AND_DISEASE_ITS_ALL_ABOUT_SEI
SELF

Reply

92. **Michael** says:
December 4, 2020 at 2:37 pm

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

Sollte da nicht “corona viruses” stehen anstatt “SARS viruses”?

Passt auch besser zum Rest des Paragraphs

“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].”

Reply

Bobby says:
December 4, 2020 at 2:44 pm

Da hast du Recht, vielen Dank für den Hinweis. Wird umgehend ausgebessert online und nachgereicht bei Eurosurveillance.

Reply

93. **Jack Leenders** says:
December 4, 2020 at 2:46 pm

Deep respect for your profound work, fingers crossed for the impact👍🤞

Reply

94. **Geam Aston Martin V8 Convertible 1978** says:
December 4, 2020 at 3:33 pm

I constantly emailed this blog post page to all my contacts, because if like to read it after that my contacts will too. https://anunturi-parbrize.ro/index.php?cauta=geam+aston+martin

Reply

95. **Hans Kleber** says:
December 4, 2020 at 4:04 pm

debunked again
https://twitter.com/MarionKoopmans/status/1333002771738611712

this time from a Virologist.

Reply

Bobby says:
December 4, 2020 at 4:38 pm

Sorry to disappoint you:

1) this mini-“thread” was not a “debunk”, it was more of an “epicleptic episode” in my very honest opinion.
2) it seems like Marion Koopmans either didn’t read our review report or has any other kind of Attention Deficit Problem – non of our concern, here is an extensive response by Kevin Mckernan:
https://threadreaderapp.com/thread/1333846936332464129.html
3) I personally can’t take anyone serious who references the low-tier “virology down under” blog by Ian M. Mackay, the analogy therefor would be: To reference Alex Jones & Infowars and declare it as a valid resource for any scientific discussion.

Reply

96. **Contra el Encierro** says:
December 4, 2020 at 7:45 pm

Dear authors,

Thank you so much for you work and for your very good idea in making this live web site (with comments): this is what is needed and this is what they do not want: an open in-depth debate. You are at the highest point of your life. Congratulations.

We have just published a full translation of your report in Spanish:
https://contraelencierro.blogspot.com/2020/12/review-report-corman-drosten-et-al.html

We will follow comments and news in your site very closely. Thanks again:

https://contraelencierro.blogspot.com

Courage!

Reply


Bobby says:
December 4, 2020 at 8:12 pm

Thank you very much, we have added it under the "spanish" sub section:
https://cormandrostenreview.com/press-voices-social-media/

Reply

97. **Georg Bauer** says:
December 4, 2020 at 8:17 pm

If the percentage of false positive with 97% is correct the whole system of political efforts in Europe and the USA may collapse.

Reply

98. **Meikel** says:
December 4, 2020 at 9:11 pm

Liebes Team!

Vielen Dank für Ihre Arbeit!
Ich hoffe sehr, dass sie zu einem kritischen Blick auf die in meinen Augen strategielose Testung bzgl. SARS-Cov2 führt.
Ich kann nicht beurteilen, ob der Corman-Drosten-Test gut oder schlecht ist. Vielleicht musste es ja wirklich schnell gehen, vielleicht auch nicht (es scheint eher Letzteres).
Prof. Kämmerer erwähnte in der Sitzung 28 (?) des Corona-Ausschuss, dass sinngemäß „ein Praktikant den Test so eben bestanden hätte".
Natürlich ist es fatal, einen Test mit der Schulnote 4- (so nenne ich da jetzt mal) einzusetzen, um solch gravierende und in die Grundrechte eingreifende Massnahmen zu begründen.
Soweit ich mich über PCRs informieren konnte, weiß ich, dass „sie quasi jede noch so geringe Kleinigkeit finden könnten", bei geringer Prävalenz mehr false-positiv generieren und zur DIAGNOSTIK nicht gedacht waren.
Trotzdem sind PCRs sicherlich sehr gut, wenn man weiß wann und wie man sie einsetzt und ebenso versteht, dass Ergebnis zu interpretieren.
Letzteres scheint mir Letzteres das derzeit das größte Problem zu sein.
Wie sollte es denn normalerweise in medizinischer Diagnostik laufen?
Der Patient geht zum Arzt, weil er „Probleme"/Symptome hat!
Der Arzt macht seine Anamnese und mögliche körperliche Untersuchungen.
Daraus entsteht eine Hypothese bzgl. der Diagnose, die womöglich noch durch technische Diagnoseverfahren (hier die PCR) unterstützt oder verworfen werden kann.
Kommt nun z.B. ein Pat. in eine Praxis und klagt über die typischen SARS-CoV2 Symptome, wäre der Arzt natürlich geneigt oder verpflichtet einen Test zur Abklärung einzusetzen.
Wählt er/sie nun die PCR, erhält er folgende mögliche Information ...
a) Test negativ, es folgt wohl die logische Erklärung „KEIN CORONA", der Pat hat eine andere Erkältungserkrankung.
b) Test Ergebnis POSITIV
b.1) Der Arzt erhält zum Ergebnis POSITIV auch den CT Wert mit z.B. 40 ...
Das Problem beginnt ... das Gesundheitsamt verzeichnet den Patienten als CORONA-

infiziert, der CT von 40 sagt aber eigentlich … nicht mehr krank oder „nur mal was abbekommen" … die Infektiösität ist laut dieser Studie (https://www.nature.com/articles/s41467-020-19802-w) quasi 0. Es folgt trotzdem QUARANTÄNE! K1 Personen desgleichen!
b.2) CT ist bei pos. Ergebnis <30 (ich bin nett und nehme den vom RKI empfohlenen Wert) … ja, hier darf man annehmen, das der Pat. wohl infiziert ist bzw. die Viruslast Massnahmen wie eine Quarantäne rechtfertigen würden.

Jeder Test ist nur so gut, wie er im Gesamtkontext eingesetzt wird. Auch ein wesentlich besser „gebauter" PCR Test kann immer noch falsch eingesetzt werden, wenn die Fragen „Wann und wen teste ich?" und „Wie interpretiere ich das Ergebnis inkl. CT?" in Bezug auf die Anamnese des behandelnden Arztes nicht bedacht werden! Hier scheinen mir noch viel größere Fallstricke zu lauern.
Problematisch an dem Corman-Drosten-Test wäre für mich auch, sollte er wirklich so „schlecht" sein, dass er als GOLD-Standard definiert ist.
Jeder eigentlich „bessere" Test wird durch seine Abweichung zum GOLD-Standard somit als schlecht bewertet.
So hätte wohl auch der AntiGen-Test eine deutlich bessere Reputation, wenn er nicht mit den Positivraten des Drosten-PCR verglichen werden würde. Dazu z.B. auch jenen Diskussion (https://infekt.ch/2020/10/covid-19-antigen-test-schlechter-als-pcr-wirklich/)oder auch die Aussagen von Frau Prof. Kämmerer im Corona-Ausschuss.
Nochmals danke ich Ihnen allen für die Zeit und Mühen die Sie in dieses Paper gesteckt haben! Diese Arbeit ist einer der letzten Hoffnungsschimmer, die ich noch habe, dass sich „die Dinge" noch ändern könnten.

Reply

99. **steve gangloff** says:
December 4, 2020 at 10:20 pm

Can you provide a PDF of this document – please forward to my email below. thanks

Reply


Bobby says:
December 5, 2020 at 5:45 am

You can get the PDF here: https://cormandrostenreview.com/downloads/

Reply

100. **Wolfgang E.** says:
December 5, 2020 at 5:09 am

Thank you for doing science!

Reply

101. **Emanuel E. Garcia, MD** says:
December 5, 2020 at 6:56 am

An exemplary scientific critique of the subject matter that deserves worldwide attention. It is abundantly clear to me that the so-called pandemic was driven by non-scientific agendas. Your analysis of the Corman-Drosten report, unlike most of what we get about COVID, represents real science. Thank you.

Reply

102. **五十嵐敬冶** says:
December 5, 2020 at 8:28 am

私も、このレポートが発表される以前に、ドロステンのRT-PCR法には「重大な欠陥」があると気付きました。
彼のプロトコルは2003年のSARSウィルス株のゲノムを参照して策定しています。
中国チームが採取したという検体のゲノムではありません。
「推測による検出方法」なのです。
それ故に、(一人でも多く「陽性者」を捕まえられるように。自説プロトコルが結果を示せるように。)必要以上にCt値(増幅サイクル数)が高く(45に)設定されているのです。

ModEdit: Translation:

Before this report was published, I also realized that Drosten's RT-PCR method had "serious flaws."
His protocol is based on the genome of the 2003 SARS virus strain.
It is not the genome of the sample collected by the Chinese team.
It is a "guessing detection method".
Therefore, the Ct value (amplification cycle number) is set higher (to 45) than necessary (so that as many "positive people" as possible can be caught. So that the self-explanatory protocol can show the result.).

Reply

103. **Charles** says:
December 5, 2020 at 11:01 am

Hi,

Thank you for the brave work you are doing, hopefully you can answer these questions i have ?

At Amphia Hospital and Bravis Hospital, total
nucleic acids were extracted for RT-PCR after an external
lysis step (1:1 with lysis binding buffer; Roche Diagnostics,
Almere, Netherlands), using MagnaPure96 (Roche) with
an input volume of 500 µL and output volume of 100 µL.

The extraction was internally controlled by addition of a
known concentration of phocine distemper virus (PDV) ??.

Is this a normal procedure that Koopmans et.al. use ?

And what are they selling at https://www.european-virus-archive.com/virus/human-2019-ncov-isolate if there is no Sars-Cov-2 isolate

https://www.european-virus-archive.com/evag-portal/evag-partners/rivm ?

Thank you and we keep monitoring them 😉

Charles..

Reply

1. **Robert** says:
December 5, 2020 at 4:28 pm

I have a hard time calling this an isolate, you?
"Culture Medium: DMEM (Dulbecco's Modified Eagles Medium) (PAA, Cölbe, Germany) with 4.5 g/L Glucose (PAA) supplemented with 10% Foetal Bovine Serum (PAA), 1% Penicillin/Streptomycin 100 x concentrate (Penicillin 10000 U/mL, Streptomycin 10 mg/ mL) (PAA), 1% L-Glutamine 200 mM, 1% Sodium Pyruvate 100 mM (PAA), 1% MEM nonessential amino acids (NEAA) 100 x concentrate (PAA) – Subculture routine: Detachment with an EDTA/trypsin mixture (PAA)"

Reply

104. **Charles** says:
December 5, 2020 at 12:05 pm

Running on 45 cycles:

https://www.researchgate.net/publication/342651957_COVID-19_in_health-care_workers_in_three_hospitals_in_the_south_of_the_Netherlands_a_cross-sectional_study

At Amphia Hospital and Bravis Hospital:
Amplification was done in a 7500SDS (Thermofisher) with a cycling profile of 5 min at 50°C, 20 s at 95°C, 45 cycles of 3 s at 95°C, and 30 s at 58°C.

At Elisabeth-TweeSteden Hospital:

Amplification with Rotorgene (QIAgen) consisted of 5 min at 50°C and 15 min at 95°C followed by 45 cycles of 15 s at 95°C, 30 s at 60°C, and 15 s at 72°C.

Partly based on these data, SARS-CoV-2 was concluded to have already spread in the population in the province of North Brabant, which led to a change of policy, in which containment measures were complemented by targeted physical distance measures, starting in the south of the Netherlands initially and later comprising the whole country.

Reply

105. **RJ** says:
December 5, 2020 at 2:22 pm

Fix your zip file. The dcox and pdf files will not extract!

Reply

Bobby says:
December 5, 2020 at 2:51 pm

Thank you for pointing out and sorry for the inconvenience. The file names in the ZIP files were too long and it didn't matter on my Mac System. This has been fixed now here:

https://cormandrostenreview.com/downloads/

And also all supplementary hyperlinks have been fixed here in the reference list:

https://cormandrostenreview.com/report/

Reply

106. **оксана** says:
December 5, 2020 at 5:08 pm

огромная благодарность за проделанную работу!теперь все пазлы и сложились.

ModeEdit: Translation:

huge gratitude for the work done! now all the puzzles have come together.

Reply

107. **Su Obreen** says:
December 6, 2020 at 7:38 am

Finally! What 'VIRUSWAARHEID.NL' has already established from the start of the COVID19 gate is now being brought to light by other celebrities / prominent figures.

Reply

108. **jac** says:
December 6, 2020 at 10:23 am

Deep respect for your work.

Reply

109. **E** says:
December 6, 2020 at 10:47 am

FYI in this article the "friend" of P Borger claim that his (Marc Bonte UmcU) rapid test has 100% specificity.

Please stop this ongoining noncense!

https://www.medrxiv.org/content/10.1101/2020.10.16.20214189v1
https://www.medrxiv.org/content/10.1101/2020.10.16.20214189v1

Reply

110. **Parbriz Skoda Rapid Spaceback Nh1** says:
December 6, 2020 at 11:55 am

Thanks for your marvelous posting! I genuinely enjoyed
reading it, you will be a great author. I will be sure to bookmark your blog and will eventually come back at some
point. I want to encourage you continue your
great work, have a nice holiday weekend! https://anunturi-parbrize.ro/index.php?page=18401

Reply

111. **ale** says:
December 6, 2020 at 1:23 pm

In the meantime it would b e unfair to all concerned to comment or discuss further untill we have looked at all the issues.(editorial note Eurosurvaillance.) Dr.Peter Borger"s opinion on that"?
And I guess you are expecting a second and about the same letter from ECDC in the very, very far future?.

Reply

Bobby says:
December 6, 2020 at 2:20 pm

Irrelevant, this was always thought as transparent and public extern review report. Otherwise it would have vanished unnoticed. Scientific additions / remarks are commented and that is an integral part of scientific discussions, that is the core reason why science has brought prosperity to mankind in the past, in the present and also in the future. Present time developments already indicate that future review processes will be transparent and most probably hard to corrupt through decentralized technology & review processes.

Further: Eurosurveillance had refused to make the review process transparent concerning the CD-paper, on two pages they explained why they can't reveal the review process protocol of the CD-paper, and none of the reasons given was in my honest opinion a valid one. In case of "fear" of revealing identities: They could have been retracted names, and the rest of the necessary information could have been made transparent & available, but they refused to do so, which is a direct anti-thesis to common scientific discussions and transparent review processes. How would you check otherwise whether a review process was valid and clean if not by revealing the review process protocol? Just by saying: "It was ok! Believe us!" ?

Further Note #2: The consequences of RT-qPCR-mass tests include the entire destroyment of whole economies, people's freedom is taken, death through restrictions, tragedies in elderly homes, ...

I think facing these consequences here, it is more than a valid step, to make the review report a) public and b) actively discuss it with the community and normal people. It is in the interest of all involved, also Eurosurveillance.

We are far beyond the tipping point here to get lost in unrelevant formalities. We are not revealing the next big thing in science here, we are discussing the flaws of currently approved publications & protocols.

If you want to educate yourself about the consequences of False Positives, head over to our guest article, with references given:
https://cormandrostenreview.com/false-positives-consequences/

I have forwarded your remarks to Dr. Peter Borger, but I think I have answered it for him – he would give you most probably the same answer.

Another fun-fact: Eurosurveillance has set-up a comments section, it's new, is it in reaction to our approach to ensure more discussion and transparency?

Reply

112. **Arturas Minajevas** says:
December 6, 2020 at 2:11 pm

Thanks alot from Lithuania!

Reply

Leave a Reply

Your email address will not be published. Required fields are marked *

Comment

Name *

Email *

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Save my name, email, and website in this browser for the next time I comment.

Post Comment

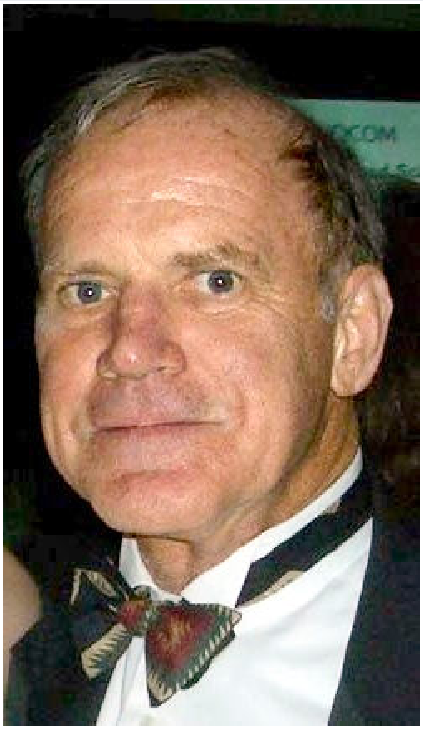
WIKIPEDIA

Bilaga 10

Kary Mullis

Kary Banks Mullis (December 28, 1944 – August 7, 2019) was an American biochemist. In recognition of his invention of the polymerase chain reaction (PCR) technique, he shared the 1993 Nobel Prize in Chemistry with Michael Smith[4] and was awarded the Japan Prize in the same year. His invention became a central technique in biochemistry and molecular biology, described by *The New York Times* as "highly original and significant, virtually dividing biology into the two epochs of before PCR and after PCR."[5]

Kary Mullis



Mullis in 2006

Born	Kary Banks Mullis December 28, 1944 Lenoir, North Carolina, U.S.
Died	August 7, 2019 (aged 74) Newport Beach, California, U.S.[1][2]
Nationality	American
Alma mater	Georgia Institute of Technology (BS, 1966) University of California, Berkeley (PhD, 1973)
Known for	Invention of polymerase chain reaction
Awards	William Allan Award (1990) Robert Koch Prize (1992) Nobel Prize in Chemistry (1993) Japan Prize (1993)[3]
	Scientific career
Fields	Molecular biology

Contents

Early life

Mullis was born in Lenoir, North Carolina, near the Blue Ridge Mountains,[6] on December 28, 1944. His family had a background in farming in this rural area. As a child, Mullis said, he was interested in observing organisms in the countryside.[7] He grew up in Columbia, South Carolina,[7] where he attended Dreher High School,[8] graduating in the class of 1962. His interest in chemistry started when he learned how to chemically synthesize and build solid fuel propulsion rockets as a high school student during the 1950s.[9]

He earned a B.S. in chemistry[6] from the Georgia Institute of Technology in Atlanta in 1966, during which time he married his first wife and started a business.[10] He earned his Ph.D. in 1973 in biochemistry at the University of California, Berkeley, in J. B. Neilands' laboratory, which focused on synthesis and structure of bacterial iron transporter molecules.[11] Although he published a sole-author paper in *Nature* in the field of astrophysics in 1968, he struggled to pass his oral exams (with a colleague recalling that "he didn't know general biochemistry"), and his dissertation was only accepted after several friends pitched in to "cut all the whacko stuff out of it" while his advisor lobbied the committee to reconsider its initial decision.[12][13]

Thesis	*Schizokinen: structure and synthetic work* (http://www.worldcat.org/title/schizokinen-structure-and-synthetic-work/oclc/17971376&referer=brief_results) (
Doctoral advisor	J. B. Neilands

Following his graduation, Mullis completed postdoctoral fellowships in pediatric cardiology at the University of Kansas Medical Center (1973-1977) and pharmaceutical chemistry at the University of California, San Francisco (1977-1979).[14]

Career

After receiving his doctorate, Mullis briefly left science to write fiction before accepting the University of Kansas fellowship.[10] During his postdoctoral work, he managed a bakery for two years.[5] Mullis returned to science at the encouragement of Berkeley friend and colleague Thomas White, who secured Mullis' UCSF position and later helped Mullis land a position with the biotechnology company Cetus Corporation of Emeryville, California.[7][5] Despite little experience in molecular biology, Mullis worked as a DNA chemist at Cetus for seven years, ultimately serving as head of the DNA synthesis lab under White, then the firm's director of molecular and biological research; it was there, in 1983, that Mullis invented the polymerase chain reaction.[15]

Mullis acquired a reputation for erratic behavior at Cetus, once threatening to bring a gun to work; he also engaged in "frequent lovers' quarrels" with his then-girlfriend (a fellow chemist at the company) and "nearly came to blows with another scientist" at a staff party.[16] According to White, "It definitely put me in a tough spot. His behavior was so outrageous that the other scientists thought that the only reason I didn't fire him outright was that he was a friend of mine."[17]

After resigning from Cetus in 1986, Mullis served as director of molecular biology for Xytronyx, Inc. in San Diego for two years. While inventing a UV-sensitive ink at Xytronyx, he became skeptical of the existence of the ozone hole.

Thereafter, Mullis worked intermittently as a consultant for multiple corporations and institutions on nucleic acid chemistry and as an expert witness specializing in DNA profiling.[14][5] While writing a National Institutes of Health grant progress report on the development of a human immunodeficiency virus (HIV) test for Specialty Labs, he became skeptical that HIV was the cause of acquired immunodeficiency syndrome (AIDS).[18] In 1992, Mullis founded a business to sell pieces of jewelry containing the amplified DNA of deceased famous people such as Elvis Presley and Marilyn Monroe.[19][20] That year, he also founded Atomic Tags in La Jolla, California. The venture sought to develop technology using atomic-force microscopy and bar-coded antibodies tagged with heavy metals to create highly multiplexed, parallel immunoassays.

Mullis was a member of the USA Science and Engineering Festival's Advisory Board.[21] In 2014, he was named a distinguished researcher at the Children's Hospital Oakland Research Institute in Oakland, California.[22]

PCR and other inventions

In 1983, Mullis was working for Cetus Corporation as a chemist.[10] While driving in the vicinity of his country home in Mendocino County with his girlfriend, who also was a chemist at Cetus, he had the idea to use a pair of primers to bracket the desired DNA sequence and to copy it using DNA polymerase; a technique that would allow rapid amplification of a small stretch of DNA and become a standard procedure in molecular biology laboratories.[10] Longtime professional benefactor and supervisor Thomas White reassigned Mullis from his usual projects to concentrate on PCR full-time after the technique was met with skepticism by their colleagues.[10][23] Mullis succeeded in demonstrating PCR on December 16, 1983, but the staff remained circumspect as he continued to produce ambiguous results amid alleged methodological problems, including a perceived lack of "appropriate controls and repetition."[10][24] In his Nobel Prize lecture, he remarked that the December 16 breakthrough did not make up for his girlfriend breaking up with him: "I was sagging as I walked out to my little silver Honda Civic. Neither [assistant] Fred, empty Beck's bottles, nor the sweet smell of the dawn of the age of PCR could replace Jenny. I was lonesome."[10]

Other Cetus scientists who were regarded as "top-notch experimentalists",[25] including Randall Saiki, Henry Erlich, and Norman Arnheim, were placed on parallel PCR projects to work on determining if PCR could amplify a specific human gene (betaglobin) from genomic DNA. Saiki generated the needed data and Erlich authored the first paper to include utilization of the technique,[5] while Mullis was still working on the paper that would describe PCR itself.[10] Mullis' 1985 paper with Saiki and Erlich, "Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia" — the polymerase chain reaction invention (PCR) — was honored by a Citation for Chemical Breakthrough Award from the Division of History of Chemistry of the American Chemical Society in 2017.[26][27]

A drawback of the technique was that the DNA polymerase in the reaction was destroyed by the high heat used at the start of each replication cycle and had to be replaced. In 1986, Saiki started to use *Thermophilus aquaticus* (Taq) DNA polymerase to amplify segments of DNA. The Taq polymerase was heat resistant and only need to be added to the reaction once, making the technique dramatically more affordable and subject to automation. This modification of Mullis' invention revolutionized biochemistry, molecular biology, genetics, medicine, and forensics. According to University of California, Berkeley biologist David Bilder, "PCR revolutionized everything. It really superpowered molecular biology—which then transformed other fields, even distant ones like ecology and evolution. ... It's impossible to overstate PCR's impact. The ability to generate as much DNA of a specific sequence as you want, starting from a few simple chemicals and some temperature changes—it's just magical."[28] Although he received a $10,000 bonus from Cetus for the invention, the company's later sale of the patent to Roche Molecular Systems for $300 million would lead Mullis to condemn White and members of the parallel team as "vultures."[10][29]

Mullis also invented a UV-sensitive plastic that changes color in response to light.

He founded Altermune LLC in 2011 to pursue new ideas on the immune system.[30] Mullis described the company's novel technology in a presentation:

> It is a method using specific synthetic chemical linkers to divert an immune response from its nominal target to something completely different which you would right now like to be temporarily immune to. Let's say you just got exposed to a new strain of the flu. You're already immune to alpha-1,3-galactosyl-galactose bonds. All humans are. Why not divert a fraction of those antibodies to the influenza strain you just picked up? A chemical linker synthesized with an alpha-1,3-gal-gal bond on one end and a DNA aptamer devised to bind specifically to the strain of influenza you have on the other end will link anti-alpha-Gal antibodies to the influenza virus and presto!--you have fooled your immune system into attacking the new virus.[6][31]

The first proof-of-principle of this technology, re-targeting pre-existing antibodies to the surface of a pathogenic strep bacteria using an alpha-gal modified aptamer ("alphamer"), was published in 2015 in collaboration with scientists at the University of California, San Diego.[32][33]

Accreditation of the PCR technique

A concept similar to that of PCR had been described before Mullis' work. Nobel laureate H. Gobind Khorana and Kjell Kleppe, a Norwegian scientist, authored a paper 17 years earlier describing a process they termed "repair replication" in the *Journal of Molecular Biology*.[34] Using repair replication, Kleppe duplicated and then quadrupled a small synthetic molecule with the help of two primers and DNA polymerase. The method developed by Mullis used repeated thermal cycling, which allowed the rapid and exponential amplification of large quantities of any desired DNA sequence from an extremely complex template. Later a heat-stable DNA polymerase was incorporated into the process.

His co-workers at Cetus, who were embittered by his abrupt departure from the company,[10] contested that Mullis was solely responsible for the idea of using Taq polymerase in PCR. However, other scientists have written that the "full potential [of PCR] was not realized" until Mullis' work in 1983,[35] and that Mullis' colleagues failed to see the potential of the technique when he presented it to them.[19] As a result, some controversy surrounds the balance of credit that should be given to Mullis versus the team at Cetus.[5] In practice, credit has accrued to both the inventor and the company (although not its individual workers) in the form of a Nobel Prize and a $10,000 Cetus bonus for Mullis and $300 million for Cetus when the company sold the patent to Roche Molecular Systems. After DuPont lost out to Roche on that sale, the company unsuccessfully disputed Mullis' patent on the alleged grounds that PCR had been previously described in 1971.[10] Mullis and Erlich took Cetus' side in the case, and Khorana refused to testify for DuPont; the jury upheld Mullis' patent in 1991.[10] However, in February 1999, the patent of Hoffman-La Roche (United States Patent No. 4,889,818) was found by the courts to be unenforceable, after Dr. Thomas Kunkel testified in the case *Hoffman-La Roche v. Promega Corporation*[36] on behalf of the defendants (Promega Corporation) that "prior art" (i.e. articles on the subject of Taq polymerase published by other groups prior to the work of Gelfand and Stoffel, and their patent application regarding the purification of Taq polymerase) existed, in the form of two articles, published by Alice Chien et al. in 1976,[37] and A. S. Kaledin et al. in 1980.[38]

The anthropologist Paul Rabinow wrote a book on the history of the PCR method in 1996 (titled *Making PCR*) in which he discussed whether Mullis "invented" PCR or "merely" came up with the concept of it. Rabinow, a Foucault scholar interested in issues of the production of knowledge, used the topic to argue against the idea that scientific discovery is the product of individual work, writing, "Committees and science journalists like the idea of associating a unique idea with a unique person, the lone genius. PCR is thought by some to be an example of teamwork, but by others as the genius of one who was smart enough to put things together which were present to all, but overlooked. For Mullis, the light bulb went off, but for others it did not. This is consistent with the idea, that the prepared (educated) mind who is careful to observe and not overlook, is what separates the genius scientist from his many also smart scientists. The proof is in the fact that the person who has the light bulb go off never forgets the "Ah!" experience, while the others never had this photochemical reaction go off in their brains."[39]

Contrarian scientific views and HIV/AIDS denial

Mullis was quoted saying "the never-ending quest for more grants and staying with established dogmas" has hurt science.[10] He believed that "science is being practiced by people who are dependent on being paid for what they are *going* to find out," not for what they actually produce.[10] Mullis was described by some as an "impatient and impulsive researcher" who finds routine laboratory work boring and instead thinks about his research while driving and surfing. He came up with the idea of the polymerase chain reaction while driving along a highway.[40]

A *New York Times* article listed Mullis as one of several scientists who, after success in their area of research, go on to make unfounded, sometimes bizarre statements in other areas.[41] In his 1998 humorous autobiography proclaiming his maverick viewpoint, Mullis expressed disagreement with the scientific evidence supporting climate change and ozone depletion, the evidence that HIV causes AIDS, and asserted his belief in astrology.[18] [42] Mullis claimed climate change and HIV/AIDS theories were promulgated by a conspiracy of environmentalists, government agencies, and scientists attempting to preserve their careers and earn money, rather than scientific evidence.[18] The medical and scientific consensus considers these hypotheses as pseudoscience, HIV having been conclusively proven to be the cause of AIDS[43][44] and global warming strongly shown to be caused by human activities.[45][46][47]

Mullis wrote that he began to question the AIDS consensus while writing a NIH grant progress report and being unable to find a peer-reviewed reference that HIV was the cause of AIDS.[18][48] He published an alternative theory of AIDS in 1994,[49] and questioned the scientific validity of the link between HIV and AIDS, leading some to label him an "AIDS denialist."[50][51] Mullis has been criticized for his association with HIV skeptic Peter Duesberg,[52] claiming that AIDS is an arbitrary diagnosis used when HIV antibodies are found in a patient's blood.[53] In 2006, Mullis wrote the foreword to the book *What If Everything You Thought You Knew About AIDS Was Wrong?* by Christine Maggiore,[48] an HIV-positive AIDS denialist who, along with her 3-year-old daughter, died of an AIDS-related illness in 2009.[54] According to journalist Coby McDonald, Mullis' HIV skepticism influenced Thabo Mbeki's denialist policymaking throughout his tenure as president of South Africa from 1999 to 2008, contributing to as many as 330,000 unnecessary deaths.[55] An article in the *Skeptical Inquirer* described Mullis as an "AIDS denialist with scientific credentials [who] has never done any scientific research on HIV or AIDS."[56] However, he consulted for Specialty Labs, in Santa Monica, developing a nucleic acid based HIV test. Seth Kalichman, AIDS researcher and author of *Denying AIDS*, "[admits] that it seems odd to include a Nobel laureate among the who's who of AIDS pseudoscientists".[57]

Use of hallucinogens

Mullis practiced clandestine chemistry throughout his graduate studies, specializing in the synthesis of LSD; according to White, "I knew he was a good chemist because he'd been synthesizing hallucinogenic drugs at Berkeley."[58] He detailed his experiences synthesizing and testing various psychedelic amphetamines and a difficult trip on DET in his autobiography.[18] In a Q&A interview published in the September 1994 issue of *California Monthly*, Mullis said, "Back in the 1960s and early 1970s I took plenty of LSD. A lot of people were doing that in Berkeley back then. And I found it to be a mind-opening experience. It was certainly much more important than any courses I ever took."[59] During a symposium held for centenarian Albert Hofmann, Hofmann said Mullis had told him that LSD had "helped him develop the polymerase chain reaction that helps amplify specific DNA sequences".[60]

Personal life

Known for his quick wit and colorful stories, Mullis enjoyed surfing and playing the guitar.[61] He married four times,[10] and had a total of three children by two of his wives. At the time of his death, he had two grandchildren and was survived by his fourth wife, Nancy Cosgrove Mullis.[62] He used profanities to make a point and often said, "Oh, Lord," in response to the absurdities experienced in life.[10] Mullis reported an encounter with a "standard extraterrestrial raccoon" at his cabin in the woods of northern California around midnight one night in 1985; he denied being on psychedelic drugs at the time.[63][18]

In a 1994 *Esquire* profile, journalist Emily Yoffe documented how Mullis (who was under the influence of alcohol) attempted to forcibly kiss her on three occasions; after the final attempt, he asked her, “How can you say you know me without sleeping with me?”[64] According to Yoffe, he also displayed photos of "women in various stages of undress" on his refrigerator door.

Mullis died of pneumonia[1] on August 7, 2019 at age 74 in Newport Beach, California.[2]

Publications and books

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- Mullis' 1998 humorous autobiography *Dancing Naked in the Mind Field* (ISBN 978-0679774006), gives his account of the commercial development of PCR, as well as providing insights into his opinions and experiences. In the book, Mullis chronicles his romantic relationships, use of LSD, synthesis and self-testing of novel psychoactive substances, belief in astrology and an encounter with an extraterrestrial in the form of a fluorescent raccoon.

Awards and honors

- 1990 – William Allan Memorial Award of the American Society of Human Genetics,[65] Preis Biochemische Analytik of the German Society of Clinical Chemistry and Boehringer Mannheim[66]
- 1991 – National Biotechnology Award,[67] Gairdner Award,[67] R&D Scientist of the Year,[67] John Scott Award of the City Trusts of Philadelphia[68]
- 1992 – California Scientist of the Year Award[67]
- 1992 – Robert Koch Prize[69]
- 1993 – Nobel Prize in Chemistry, Japan Prize,[3] Thomas A. Edison Award[67]
- 1994 – Honorary degree of Doctor of Science from the University of South Carolina[22]
- 1994 – Golden Plate Award of the American Academy of Achievement[70]
- 1998 – Inducted into the National Inventors Hall of Fame,[71] Ronald H. Brown American Innovator Award[72]
- 2004 – Honorary degree in Pharmaceutical Biotechnology from the University of Bologna, Italy[73]
- 2010 – Honorary degree of Doctor honoris causa in the field of biological sciences from Masaryk University, Czech Republic[74]

See also

- House of Numbers
- Luc Montagnier – Nobel prize-winning HIV researcher
- Nobel Prize controversies

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